UNIV. OF

1921

Digitized by the Internet Archive
in 2010 with funding from
University of Toronto

http://www.archive.org/details/collectedpapers05univ

London, University of.

UNIVERSITY COLLEGE HOSPITAL

MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

Graham

RESEARCH DEPARTMENT

Directors :

Dr. CHARLES BOLTON.

Dr. A. E. BOYCOTT, F.R.S.

COLLECTED PAPERS

160702
8/4/21

VOL. V.—JAN., 1916.

R
111
L7
v.5-6

CONTENTS.

1. The effect of removal of large portions of renal substance on the heart. By F. J. F. **Barrington**. *Heart*, Vol. vi., (1915).

2. The mucinous changes of the vaginal epithelium of certain mammals in pregnancy. By F. J. F. **Barrington**. *Journal of Anatomy and Physiology*, vol. l., (1915), p. 30.–36 n.p

3. The part played by the acid of the gastric juice in the pathological processes of gastric ulcer. By C. **Bolton**. *Journal of Pathology and Bacteriology*, vol. xx., (1915), p. 133–158 3 plates 15

4. Some results of excision of the adrenal glands. By T. R. **Elliott**. *Journal of Physiology*, vol. xlix., (1914), p. 38–

5. The relations between successive responses of the ventricle to electrical stimulation and ventricular fibrillation. By S. G. **Levy**. *Journal of Physiology*, vol. xlix., (1914), p. 54–66 n.p

6. Notes of further observations upon dyspusea and its relation to blood reaction. By T. **Lewis** and J. **Barcroft**. *Quarterly Journal of Medicine*. vol. viii., (1915), p. 97–113 n p

7. The excitatory process of the dog's heart; part 2, the ventricle. By T. **Lewis** and M. A. **Rothschild**. *Philosophical Transactions*, B. vol. ccvi., (1915), p. 181–226
4 plates with extra

8. Lipoids in immunity. By F. H. **Theile** and D. **Embleton**. *Journal of Pathology and Bacteriology*, vol. xix., (1915), p. 349–371

9. The mechanism of antibody action. By F. H. **Thiele** and D. **Embleton**. *Journal of Pathology and Bacteriology*, vol. xix., (1915), p. 372–

10. *Klossiella muris.* By A. C. **Stevenson**. *Quarterly Journal of Microscopical Science*, vol. xli., (1914), p. 127–135 with

Hs 1.
63

[*Reprinted from "Heart,"*
Vol. VI., No. 2, October, 1915.]

THE EFFECT OF REMOVAL OF LARGE PORTIONS OF THE TOTAL RENAL SUBSTANCE ON THE HEART.[*]

By F. J. F. BARRINGTON.

(*From the Graham Research Laboratories, University College Hospital Medical School.*)

THE following experiments were made to determine whether the mere absence of a large fraction of the total renal substance produced an enlargement of the heart at all comparable to that seen in man in certain forms of renal disease. Cats were used exclusively. The normal range of variation in the size of the heart and kidneys was found by an examination of a hundred individuals of each sex. The hearts were weighed empty of blood and were cut out at the reflexion of the pericardium off the great vessels. The renal vessels were cut as close to the hilus as possible and the true capsules were weighed with the kidneys. Neither castrated males nor pregnant females were included, the former were found to have proportionally much smaller hearts and kidneys than normal males. The following table gives the results ; the weights are in grammes.

	Weight.	Heart.		Both kidneys.	
		Weight.	% of body.	Weight.	% of body.
			Males.		
Maximum	4280	20·33	0·566	62·05	1·748
Minimum	1980	8·17	0·336	16·78	0·628
Mean	3158	13·74	0·435	35·75	1·126
			Females.		
Maximum	3450	17·87	0·712[†]	46·05[‡]	1·507[‡]
Minimum	1835	6·53	0·295	12·17	0·557
Mean	2605	10·52	0·404	22·34	0·858

Ether was the anæsthetic used for all operations. Each experiment consisted in first removing a wedge from the upper pole of one kidney and subsequently, usually after an interval of three weeks, removing the remaining kidney or tying its ureter.

---

[*] The expenses of this research were defrayed by a Beit Memorial Fellowship and grants from the Graham Research Fund.

[†] The next lower number in the series was 0·545%.

[‡] Both figures came from the same cat which, though in apparent health, had diseased kidneys. The corresponding figures next below were 42·78 gr. and 1·314% which were also from one cat.

Fifteen experiments were made of which the following are the summaries.

1. Male. September the 14th, 1911. 2780 gr.. Upper pole of left kidney removed.

September the 28th, 1911. 2495 gr.. Right kidney removed, weight 11·42 gr..

October the 24th, 1911. Dead, appeared well yesterday. Weight 2400 gr. kidney 18·48 gr., heart 0·500 gr.. Kidney somewhat hydronephrotic.

2. Male. October the 30th, 1911. 3200 gr.. Upper pole of left kidney removed, weight 3·03 gr..

November the 21st, 1911. 3490 gr.. Right kidney removed, weight 17·40 gr..

February the 5th, 1913. 3450 gr.. Killed. Heart weighed 17·2 gr. or 0·509% of body weight. Kidney 24 gr. or 0·696% of body. Carotid blood-pressure under ether 160 mm. Hg..

3. Male. October the 30th, 1911. 3425 gr.. Upper pole of left kidney removed, weight 3·29 gr..

November the 21st, 1911. 3650 gr.. Right kidney removed, weight 21·30 gr..

July the 26th, 1912. 2900 gr.. Died with nasal discharge and enteritis. Heart weighed 16·35 gr., kidney 27 gr..

4. Female. December the 1st, 1911. 2325 gr.. Upper pole of left kidney removed, weight 2·00 gr..

December the 22nd, 1911. 2170 gr.. Right kidney removed, weight 9·97 gr..

January the 13th, 1913. 3110 gr.. Killed. Heart weighed 12·8 gr. or 0·412% of body weight. Kidney 15·77 gr. or 0·504%. Carotid blood-pressure under ether 180 mm. Hg..

5. Male. December the 1st, 1911. 3570 gr.. Upper pole of left kidney removed, weight 4·22 gr..

December the 22nd, 1911. 3050 gr.. Right kidney removed, weight 15·56 gr..

June the 4th, 1912. 2800 gr.. Killed. Heart weighed 10·13 gr. or 0·365% of body weight. Kidney 17·35 gr. or 0·619% of body.

6. Female. March the 29th, 1912. Upper pole of left kidney removed, weight 1·95 gr..

April the 19th, 1912. 2160 gr.. Right kidney removed, weight 13·37 gr..

April the 20th, 1913. 1990 gr.. Killed. Heart weighed 7·30 gr. or 0·367% of body weight. Kidney 12·11 gr. or 0·609% of body.

7. Male. January the 22nd, 1913. 2735 gr.. Upper pole of left kidney removed, weight 2·17 gr..

February the 2nd, 1913. 2675 gr.. Right ureter tied.

February the 23rd, 1914. 3900 gr.. Killed. Heart weighed 13·92 gr. or 0·357% of body weight. Kidney (left) 34·55 gr. or 0·809% of body. Carotid blood-pressure under ether 200 mm. Hg.. Right kidney a hydronephrotic sac.

8. Male. September the 22nd, 1913. 3500 gr.. Upper pole of left kidney removed, weight 4·31 gr..

October the 12th, 1913. 3300 gr.. Right ureter tied.

October the 22nd, 1913. 2620 gr.. Killed. Not feeding well some days. Heart weighed 11·65 gr.. Left kidney 12·13 gr.. No hydronephrosis of the right kidney.

9. Female. September the 22nd, 1913. 2325 gr.. Upper pole of left kidney removed, weight 2·67 gr..

October the 12th, 1913. 2500 gr.. Right kidney removed, weight 9·31 gr..

November the 17th, 1913. 1520 gr.. Dead. Not feeding well four weeks. Heart weighed 6·93 gr.. Kidney 7·16 gr..

10. Female. September the 22nd, 1913. 2635 gr.. Upper pole of left kidney removed, weight 2·44 gr..

October the 12th, 1913. 2525 gr.. Right ureter tied.

December the 2nd, 1913. 1750 gr.. Dead. Nasal discharge. Heart weighed 8·34 gr.. Left kidney 12·91 gr.. Right kidney moderately hydronephrotic.

11. Female. September the 30th, 1913. 3175 gr.. Upper pole of left kidney removed, weight 3·96 gr..

October the 21st, 1913. 3120 gr.. Right kidney removed, weight 17·01 gr..

April the 18th, 1914. 3850 gr.. Dead. Full term pregnancy. Weight without pregnant uterus 2725 gr.. Heart weighed 13·49 gr.. Kidney 19·30 gr..

12. Female. September the 30th, 1913. 2950 gr.. Upper pole of left kidney removed, weight 3·50 gr..

October the 21st, 1913. 2700 gr.. Right kidney removed, weight 15·62 gr..

November the 12th, 1913. 1650 gr.. Dead. Heart weighed 8·04 gr.. Kidney 8·27 gr..

13. Female. September the 30th, 1913. 2375 gr.. Upper pole of left kidney removed, weight 2·58 gr..

October the 21st, 1913. 2460 gr.. Right kidney removed, weight 11·37 gr..

November the 22nd, 1913. 1570 gr.. Dead. Heart weighed 6·12 gr.. Kidney 9·43 gr..

14. Female. September the 30th, 1913. 2075 gr.. Upper pole of left kidney removed, weight 2·50 gr..

October the 21st, 1913. 2050 gr.. Right ureter tied.

March the 18th, 1914. 2900 gr.. Dead. Pregnant nearly full term. Weight without pregnant uterus 2350 gr.. Heart weighed 9·86 gr.. Left kidney 13·93 gr.. Right kidney a hydronephrotic sac.

15. Female. December the 4th, 1913. 3480 gr.. Upper pole of left kidney removed, weight 3·64 gr..

December the 31st, 1913. 3510 gr.. Right ureter tied.

February the 8th, 1915. 3180 gr.. Killed. Heart weighed 14·43 gr. or 0·454% of body weight. Left kidney 20·34 gr. or 0·679% of body. Right kidney a hydronephrotic sac.

All the cats were kept in a large cage during a considerable part of each experiment, so that they got a fair amount of exercise.

It will be seen that in five cases (1, 8, 9, 12 and 13) the well-known wasting symptoms produced by the removal of an excessive proportion of the total renal substance developed. In two (3 and 10) the cats died with an accidentally acquired, wasting, infectious disease. It is evident that no conclusions involving the relative size of the heart can be drawn from any of these seven experiments, since in emaciation the heart is known to waste less than the whole body. Two (11 and 14) became pregnant months after the operation and died at full term : it is not known what symptoms they exhibited but both appeared quite well the day before death. It appears possible that, though the remaining amount of kidney was sufficient to keep them in health in ordinary circumstances, it was insufficient in the later stage of pregnancy. Neither of these cats can be considered for the present purpose, as the normal relative size of the heart in pregnancy was not determined.

There remain six cats (2, 4, 5, 6, 7 and 15), three of either sex, which remained healthy till killed. In these the percentage weight of heart to body was well within the normal limits. The mean in males was 0·407 % and in females 0·411 %, the former being below and the latter above the normal mean.

It may therefore be concluded that the removal of such fractions of the total renal substance as are compatible with prolonged life produces no cardiac hypertrophy in cats.

THE MUCINOUS CHANGES OF THE VAGINAL EPITHELIUM OF CERTAIN MAMMALS IN PREGNANCY.[1] By F. J. F. BARRINGTON.

(From the Graham Research Laboratories, University College Hospital Medical School.)

THE tissues of freshly killed animals were hardened in a sublimate-formol-acetic mixture and cut in paraffin. Mallory's iron hæmatoxylin, van Gieson and Mayer's muci-carmine, were the stains used. The latter was the only mucin reagent employed.

GUINEA-PIG.

In Pregnancy and Lactation.—Only such animals were considered pregnant as showed visible swellings in one or both uteri. It therefore follows that the very earliest stages of pregnancy were not observed in this series, since the uterine swelling takes some time to develop. The period of pregnancy was not known in any case; it was estimated roughly by the size of the fœtus. Eighteen animals were examined, which were classed as five early pregnancies, six about mid-term, and seven late pregnancies. Of the last, two appeared to be about full-term. Considering first the five early cases, the vaginal epithelium of one consisted of two or three layers of cells with large, clearly staining nuclei. The cells next the lumen were shortly columnar with the nucleus situated at the basal end, while the part of the cell on the luminal side of the nucleus gave a fair mucin reaction. The epithelium of the next two cases resembled the first, except that the cells next the lumen were taller and the mucin reaction was more marked. The mucin was still only situated on the luminal side of the nucleus, so that there was not a row of nuclei in the red zone of mucin. The fourth case showed the luminal row of cells to be taller still; their nuclei were darker stained than in the basal layers, and mucin was present on both sides of the nucleus, so that there was a red zone with a row of nuclei just within it. In none of these four cases was the epithelium thrown into folds. In the fifth case the luminal row of cells was both taller and broader; the nuclei were situated near the middle of the cells, stained deeply, and were irregular in shape as if from compression. The mucous membrane was thrown into small folds and had

---

[1] The expenses of this research were defrayed by grants from the Graham Research Fund.

the appearance of being too large for the submucous layer. The cases in this class, formed of all the early pregnant guinea-pigs, thus present a series showing a gradual increase in the size of the luminal row of cells and in the amount of mucin they contain.

The six members of the second class resembled the fifth case fairly closely. In two of them the folds of the mucous membrane were much more marked and resembled those of the last class. The mucous membrane of the seven cases of late pregnancy were identical in every respect. The folds were so numerous that they had become contiguous, producing the appearance of an epithelium of eight or more layers of cells. When carefully examined, however, the epithelium was seen to be composed of the same cells as in the first stage—namely, a basal layer of one or two rows of very flattened cells with clear nuclei and no mucin, and a luminal row of tall expanded cells, greatly distended with mucin and having deeply stained, compressed nuclei situated about the centre.

From these facts it may be concluded that in guinea-pigs during pregnancy the vaginal epithelium gradually becomes both thicker and broader from an increase in size of the cells in the row next the lumen owing to their distention with mucin. The process appears to be complete some time before term in the latter half of pregnancy.

Twelve guinea-pigs have been examined at known intervals after parturition. In all twelve the young lived and went on sucking till the mothers were killed. In one, less than twenty hours after parturition, the vaginal epithelium resembled that described at full term in every respect except that the basal, non-mucinous cells were not so flattened, so that this layer was more conspicuous. Two were examined five days after parturition. In these the folds of mucous membrane showed intervals between them as if they had shrunk away from one another; the mucinous layer was rather thinner, and in it were numerous clear spaces, many of which contained leucocytes. Two cases, ten days after parturition, differed from each other. One showed simply a more advanced vacuolation and thinning of the mucinous layer than the five-day cases; the other resembled the early pregnancy cases, the epithelium having two to three layers of cells, those next the lumen being columnar loaded with mucin, and, having their nuclei near the middle, small folds were present. Three cases, fifteen days after parturition, showed thinning and vacuolation of the mucinous layer; but in one it was not more marked than in the ten cases. One case, twenty days after parturition, had an epithelium completely free from mucin: it was stratified in the ordinary way four to six cells deep; all the cells contained nuclei, and leucocytes were present between some of the cells in the layer next the lumen. Three cases were examined respectively thirty, forty, and fifty days after parturition; they all showed a mucinous layer next the lumen with advanced thinning and vacuolation. Folds were fairly well marked in the thirty-day case, but absent in the other two.

These observations, though not constant enough to draw any definite conclusions, show that soon after parturition the mucin begins to disappear. The rate of disappearance either varies considerably in individual cases or some other process occurs in the epithelium, apart from pregnancy, and becomes superimposed at varying intervals of time after. It further appears that usually, under natural conditions of lactation, the mucin is not cast off *en masse*: in only the twenty-day case could this have occurred in the twelve above described. In a guinea-pig killed six days after parturition, the young having died after one day, the vaginal epithelium was stratified and free from mucin as in the twenty-day case. Still adhering to the epithelium in some places and quite free from it in others was the whole thick mucinous layer with a layer of leucocytes between it and the epithelium. The mucinous layer was very thick and not greatly vacuolated, so it seems that in this case the basal, non-mucinous cells must have proliferated as soon as lactation ceased and the separation of the mucinous layer have been brought about by the layer of leucocytes.

In Non-pregnant Guinea-pigs.—Forty-four guinea-pigs which had no visible uterine enlargements were examined. None of them were known to have recently given birth, and in none was the uterus subinvoluted. It is probable that among the forty-four cases there were a few which were pregnant at too early a stage to give uterine enlargements, and possible that parturition had occurred in others a week or more before. The vaginal epithelium in these forty-four cases showed great variations, and all stages described as occurring in pregnancy and the puerperium were found except that seen in the last half of pregnancy. The cases fell into five groups:—

Group 1 (seven cases).—The epithelium consisted of three to five layers of cells. The cells in the layer next the lumen were distended with mucin, and this layer was thrown into folds; the folds were contiguous, giving rise to the appearance of several layers of mucinous cells. These seven cases were those which most closely resembled the condition seen in the last half of pregnancy; they differed from it in the smaller development of the mucinous layer, and in the greater number of basal layers free from mucin.

Group 2 (sixteen cases).—The epithelium was stratified and consisted of four to six layers. Mucin was either completely absent or a very faint pink tinge was seen in some of the flattened cells next the lumen.

It is quite clear that the condition seen in Group 1 is the stage which precedes that seen in Group 2, the latter being produced from the former by the casting off *en masse* of the mucinous layer. Group 1 contains cases where this layer is cast off in places and adherent in others, and Group 2 contains cases where the whole mucinous layer is cast off and lying free in the vaginal lumen.

Group 3 (six cases).—The epithelium was stratified, consisting of three to five layers of cells. The row of cells next the lumen gave a well-marked mucin reaction.

This group only differs from the last in the epithelium being rather thinner and in the presence of mucin in the luminal layer.

Group 4 (nine cases).—These exactly resembled those described already as being seen in early pregnancy. The epithelium consisted of one or two rows of cells without mucin situated basally, with a columnar row, giving a marked mucin reaction, next the lumen; folds were absent or ill-developed.

In certain cases Group 4 appeared to shade gradually into Group 3 on the one hand, and into Group 1 on the other.

Group 5 (six cases).—The epithelium resembled that already described as occurring in the puerperium. The epithelium consisted of one or two rows of mucin-free cells situated basally, with a row of mucinous cells next the lumen; folds were well marked but not contiguous, and marked cystic formation was present in the mucinous layer. Some cases in this group appeared somewhat similar to those of Group 4.

From an examination of this series it seems that the cycle passes successively through Groups 1, 2, 3, and 4 in that order and then back to Group 1. Group 5 appears also to arise from Group 3 and to show a second way in which the epithelium loses its mucin, but this, though apparently the usual way in the puerperium, is less common apart from pregnancy than the mucin being thrown off *en masse*.

It seems probable that the changes in the non-pregnant animal have some connexion with the œstrous cycle, as I have shown to be the case with the mucin in the cat's Bartholin's gland (*Internat. Monatsschr. f. Anat. und Phys.*, Bd. xxx. p. 1). Examination of the uteri and ovaries, however, failed to give confirmatory evidence of this. The mere presence of mucin does not appear to be dependent on the ovaries, since it can be found in the luminal layer of cells in the full-term guinea-pig fœtus. In four guinea-pigs which were known to have littered, both ovaries were removed and the animals killed four, four, nine and twelve months after respectively. The vaginal epithelium consisted of two rows of cells, a basal, compressed layer, free from mucin, and a columnar layer with mucin next the lumen. In one of those killed after four months, well-marked

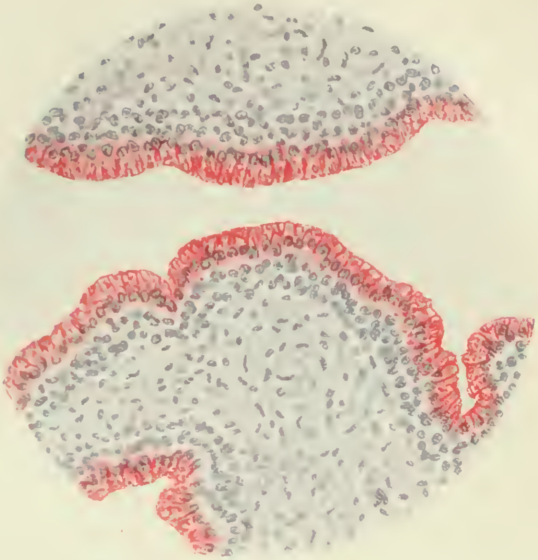


Fig. 1.—Vaginal mucous membrane of a guinea-pig during early pregnancy. (Drawn from a Leitz No. 6 objective and No. 3 eyepiece.)

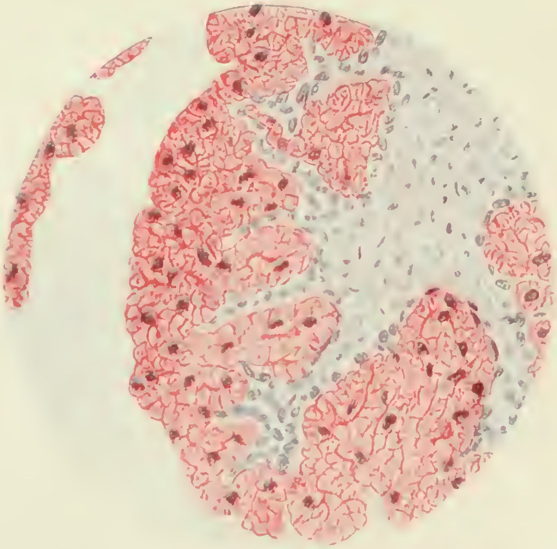


Fig. 2.—Vaginal mucous membrane of a guinea-pig pregnant at term. (Drawn from a Leitz No. 6 objective and No. 3 eyepiece.)

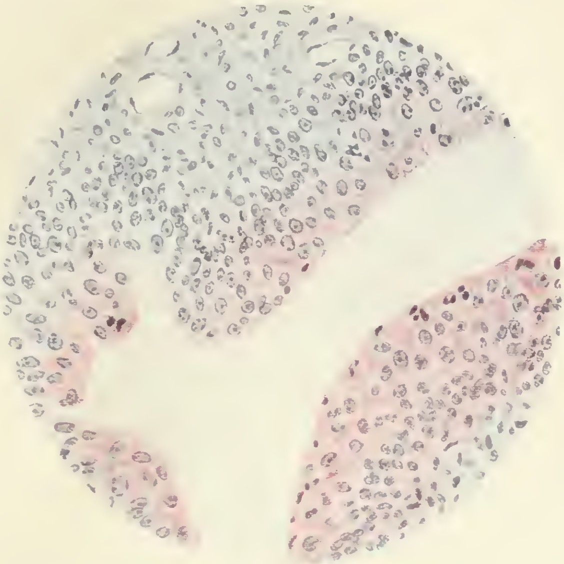


FIG. 3.—Vaginal mucous membrane of a guinea-pig twenty days after parturition. (Drawn from a Leitz No. 6 objective and No. 3 eyepiece.)

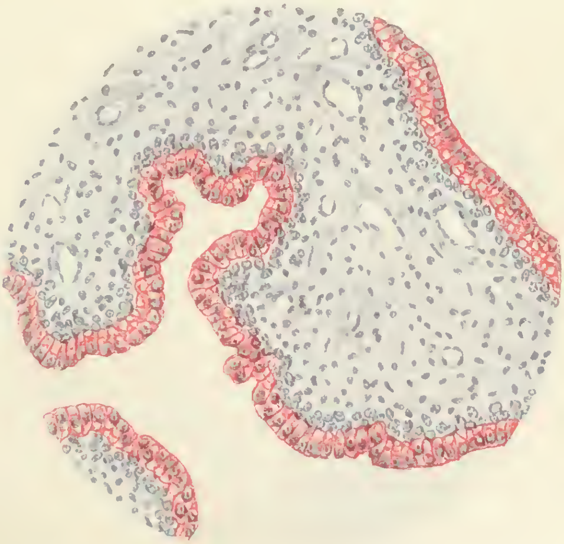


FIG. 4.—Vaginal mucous membrane of a guinea-pig one year after double ovariotomy. (Drawn from a Leitz No. 6 objective and No. 3 eyepiece.)

folds were present, but not in the three others, which resembled each other closely.

OTHER ANIMALS.

In the rat six individuals were examined. In three which were not pregnant the vaginal epithelium was of the usual stratified form and gave no mucin reaction. In one pregnant at term and another killed within twenty-four hours of parturition the vaginal epithelium consisted of one or two rows of flattened cells, free from mucin and situated basally, surmounted by about six rows of cells distended with mucin. In these sections it was not clear that the thickness of the mucinous layer was due to reduplication of the mucous membrane. The sixth rat was killed about a week after parturition, the young having died on the first day: the epithelium was stratified with four to six rows of cells: the row of flattened cells next the lumen gave a very faint mucin reaction.

Seven rabbits were examined. One had littered one to two days before and another was twenty-two days pregnant. In these two the vaginal epithelium consisted of two rows of cells. Those next the lumen were tall, columnar, full of mucin, and had the nuclei near the bases. The basal cells were very flattened and much fewer in number than those in the luminal row; they were free from mucin. In the five others, which were not pregnant, the epithelium was stratified in three to five rows. One contained no mucin: in one the luminal row was rich in mucin, and columnar; in the remaining three there was a small amount of mucin in the luminal row.

As far as pregnancy is concerned, the rat and rabbit appear to resemble the guinea-pig in the changes which take place in the vaginal epithelium.

In cats four individuals were examined. Two were in the latter half of pregnancy. The stratified epithelium had flattened cells next the lumen, a few of which gave a faint mucin reaction: mucin was present in some of the vaginal crypts. Parturition had occurred in the two others recently; in one less than twenty-four hours before death. In these no mucin was found in the epithelium.

Four hedgehogs were examined. One was in the latter half of pregnancy. The other three, which were not pregnant, were killed respectively in January, May, and September. No mucin was present in the vaginal epithelium in any case.

In the three rodents examined, therefore, a marked change takes place in the vaginal epithelium in pregnancy. This change consists in a great increase in the size of the cells next the lumen owing to their distention with mucin. This change does not occur in the cat or the hedgehog.

Journal of Pathology and Bacteriology, Vol. XX.

THE PART PLAYED BY THE ACID OF THE GASTRIC JUICE IN THE PATHOLOGICAL PROCESSES OF GASTRIC ULCER.[1]

By Charles Bolton, M.D., D.Sc., F.R.C.P., *Physician to Out-patients, University College Hospital.*

Graham Research Laboratories, University of London.

(Plates XVI.–XVIII.)

An ulcer of the stomach originates in many different ways, and the processes giving rise to it are similar to those which are responsible for the formation of ulcers in other parts of the body. The ulcer whilst in its acute stage usually tends to heal in the normal manner; occasionally the healing is delayed for varying periods of time; and in a considerable number of cases it is completely arrested. This arrest in the healing brings about the condition known as chronic gastric ulcer. The persistent tendency of chronic gastric ulcer to remain unhealed is one of the most important problems in the pathology of the disease which requires solution.

An ulcer of the stomach, although formed in a similar manner to other ulcers, differs from them in that it is exposed to certain adverse influences during the process of gastric digestion. The *normal* gastric processes cannot exert much deterring influence upon the healing of an ulcer, because most acute gastric ulcers heal rapidly, and similarly all experimenters agree that the ulcers they have formed in animals by various means have healed equally well. However, in the case of chronic gastric ulcer, the digestive processes are *not* conducted normally, and it is an everyday experience that the subjects of this disease suffer from gastric disorders of both motion and secretion.

It is practically certain that all patients with chronic gastric ulcer suffer from attacks of food retention owing to pyloric spasm or muscular insufficiency. For such a condition to produce decidedly adverse effects it is not necessary for it to be prolonged or of a high degree. Although most of the meal may leave the stomach in the normal time, certain articles of diet are not uncommonly retained and subsequently found in the stomach mixed with almost pure gastric juice or with the next meal. The ordinary bismuth meal is no test of these slighter forms of intermittent retention.

---

[1] [Received July 10, 1915.] The expenses of this research have been defrayed by grants from the Royal Society and the Graham Research Fund.

Again, it has been proved that the majority of cases of chronic gastric ulcer are associated with hyperchlorhydria, and often with hypersecretion also. It is true that in a minority the percentage of HCl is found to be normal or even diminished, but this is no proof that there is not or has not been intermittent hyperchlorhydria, particularly as repeated examination of the gastric contents in any given case of gastric ulcer is not a usual procedure. If it is permanently diminished in some cases of gastric ulcer, particularly those in which the disease is of very long standing, it must be remembered that such an ulcer owing to its chronicity may be easily affected by a low degree of acidity. One of the most important points, therefore, in the pathology of gastric ulcer involves the consideration of the relation of such disorders of motion and secretion to the various pathological processes occurring in this disease.

It has naturally been considered, for many years past, that the gastric juice played an important part either in the initiation or propagation of gastric ulcer, but this idea has been held in a vague kind of way and has not been supported by any very definite evidence with regard to what precise effects this secretion produces, and what is its mode of action.

Katzenstein (1908[1]), from his experiments, concluded that the *ferment* of the gastric juice was the chief destructive agent, and that this secretion could digest the wall of the stomach, because the latter was deficient in protective anti-ferment, but more recently Wolff (1909–10[2]) has shown that the stomach, in cases of gastric ulcer, is as rich in anti-ferment as the normal stomach, and that there is no diminution in the anti-ferment content of the blood in this disease.

For some time past I have maintained that it is the *acid* of the gastric juice which is the chief damaging factor, and from my experiments have drawn the following conclusions (1907–10[3]):

1. The normal gastric juice is able to attack the gastric mucous membrane which has been damaged or devitalised to a certain extent by a blood poison, the actual death of the cell not being a necessary antecedent to such attack.

2. This property it owes to the HCl contained in it, which completes the death of the tissue and thus allows of its digestion. The HCl acts in this capacity as a protoplasmic poison.

3. The ulceration produced in the presence of a hyperacid gastric juice (0·25 per cent. and upwards) is much more extensive than that produced in a stomach secreting normal juice.

4. Acute ulcer is more easily and quickly produced in the digesting than in the resting stomach, and is more extensive in the former than in the latter.

5. Motor insufficiency of the stomach, leading to undue retention

of food, delays the healing of an ulcer for at least twice the normal time, owing to necrosis of the granulation tissue of the base of the ulcer by the prolonged action of the HCl in the gastric contents.

6. For the same reason the healing time is prolonged by a diet which causes considerable secretion of gastric juice and leaves the stomach slowly; it is hastened by the opposite kind of diet.

Other experimenters have approached the question of the effects of hyperacidity upon the healing of the ulcer, but their deductions from the results of their experiments have not been in agreement, and the experiments themselves were not systematically conducted but were merely isolated observations.

Matthes (1893 [4]) produced a lesion in the gastric mucous membrane 6 cms. in diameter, in each of three dogs. In one animal at the end of fourteen days he found a small ulcer 2·5 cms. in diameter. In another at the end of a month a scar was present, the lesion having completely healed. The third animal was fed with 0·5 per cent. HCl solution, and at the end of three weeks an ulcer the size of a split pea with hard edges was found on killing the animal. Litthauer (1909 [5]) produced ulcers in four animals. Into one of them he repeatedly injected pyrogallic acid and into the other three pyridin, in order to produce artificial anæmia. Each dog was fed with 0·37 to 0·5 per cent. HCl solution. The animals were killed on the twelfth, forty-ninth, fifty-ninth, and sixty-third days, and in each case the ulcer was found to be healed. The days on which these ulcers healed were not ascertained. In another experiment he tied off the vessels supplying a third of the stomach with blood and fed the animal on 0·37 per cent. HCl solution. At the end of a month a deep ulcer was found in the stomach. Suzuki (1912 [6]) produced an ulcer in a dog and fed it on 0·37 per cent. HCl solution. At the end of six weeks he found a scar in the stomach.

Litthauer's experiments dealt with the effects of anæmia and hyperacidity, so there are left two experiments only which were performed to test the effects of simple hyperacidity. In one a small healing ulcer was found at the end of three weeks, and in the other a scar at the end of six weeks. We thus see that no systematic experiments have hitherto been performed in regard to the effects of hyperacidity upon the healing of ulcers.

The present research was undertaken in continuation of my former experiments and refers particularly to the effects of hyperacidity alone and combined with motor insufficiency upon the healing of gastric ulcer, and to the effects of hyperacidity of the gastric juice upon the mucous membrane of the stomach.

The subject will be considered under the following headings:

I. The effects of hyperchlorhydria upon the healing of gastric ulcer. II. The effects of hyperchlorhydria, together with motor insufficiency, upon the healing of gastric ulcer. III. The effects of hyperchlorhydria upon the gastric mucous membrane.

I.

THE EFFECTS OF HYPERCHLORHYDRIA UPON THE HEALING OF GASTRIC ULCER.

The animals used were monkeys, and a few experiments were done upon cats. In all cases the ulcers were produced by the local injection of a gastrotoxic serum into the wall of the stomach. The production of such an immune serum and the formation and normal healing of the resulting ulcers in the case of the cat have already been described in a former paper (1910 [3]), and need not now be referred to. As this is the first time a gastrotoxic serum active against the monkey has been produced, I will give a short description of the process and of the formation and healing of the resulting ulcers.

The monkey was selected as the experimental animal on account of the very close resemblance which its stomach bears to that of man. The texture of the stomach wall is more delicate than that of the cat and the muscular coat is less powerful. Anatomically the position, shape, and connections of the stomach are the same as in man. The body of the stomach is vertical and turns sharply to the right at the pyloric end, the small curvature being bent at about a right angle. The uppermost portion of the fundus contains an air space. The three portions of the duodenum have also the same anatomical relation as those of the human subject. The percentage of hydrochloric acid in the gastric contents is about 0·15 to 0·2. The diet of the monkey was in all cases a vegetable one.

Production of a gastrotoxic serum active against the monkey.—The serum was produced by the repeated injection of a fresh extract of the gastric cells of the normal monkey into the goat. The stomach of one monkey was used for each injection, and subcutaneous injections were made at intervals of about ten to fourteen days. The stomach was cut out immediately after death and thoroughly washed. The mucous membrane was then scraped off on a porcelain plate with a knife, and ground up with sterilised sand in a glass mortar. An emulsion with salt solution was thus made and centrifugalised, the supernatant fluid amounting to about 50 c.c. being used for injection. All the apparatus was of course thoroughly sterilised before use. In this way three goats were immunised and the blood serum of each was found to possess strong gastrotoxic properties for the monkey's stomach. The monkeys used were chiefly *Macacus rhesus*, but also included bonnets, two young baboons, *Patas cercopithecus*, and green *cercopithecus*. There was no specificity for any definite species of monkey; so long as the animal was a monkey its stomach was susceptible to the action of the serum. The goats were bled from a vein of the ear if small amounts of serum were required, and from the jugular vein if 100 c.c. or more were to be removed. The blood was whipped and centrifugalised at once, and the serum used for injection whilst quite fresh. After about six injections the serum is ready for use. By continued injections the goat will yield a toxic serum for many months; in one case the serum was highly toxic nine months after the commencement of the injections. If the injections are stopped the toxicity of the serum soon falls. The best time to bleed the goat is about seven to ten days after the last injection.

Injection of the serum.—The blood serum was in all cases injected into the wall of the stomach on the anterior surface at about the junction of the cardiac and pyloric portions. The monkey, which had been fed about one hour previously so that digestion was proceeding, was anæsthetised with ether and the stomach drawn out of a median incision through the abdominal wall.

The point of the needle was introduced between the coats of the stomach and from 6 to 10 c.c. of serum according to its strength injected with a glass syringe, so as to produce a round patch of œdema in the stomach wall. The abdominal wound was then closed. Iodine was used to disinfect the skin after shaving, and the operation was performed under strict aseptic conditions.

Formation and healing of the ulcer.—The normal goat's serum is without action when injected in such doses into the stomach wall of the monkey. In the case of gastrotoxic serum, however, the gastrotoxin is abstracted from the serum and becomes fixed on to the tissues, more particularly the cells of the mucous membrane. The affected portion of the mucous membrane is attacked by the gastric juice and a slough is formed, which gradually separates with the formation of a cleanly punched-out ulcer, extending to various depths in proportion to the strength of the serum; perforation may occur. The ulcer is formed in about four or five days.

The stages in the healing were worked out in a series of monkeys, and the process found to be the same as in the cat, so that a very short description will suffice. When the sloughs have separated from the edges, but before they have completely separated from the base of the ulcer, the glandular cells at the edges begin to proliferate and cover the raw surface with a single layer of flat cells. These cells become cubical in shape, and from them simple tubes, which eventually form new glands, bud off and grow down into the granulation tissue. The base of the ulcer becomes covered with granulation tissue when all the sloughs have separated, and the single layer of flat epithelial cells at the periphery grows out over this tissue, and by about the twenty-first day has completely covered it. During this time the edges of the ulcer are being drawn nearer together by the contraction of the newly formed fibrous tissue in the base of the ulcer, so that an increasingly smaller surface remains to be covered. The epithelial cells covering the base now send out buds in the shape of simple tubes which proliferate in the underlying cellular stroma, and so the new mucous membrane gradually thickens as the glands become fully formed.

The following experiments illustrate the various stages in the evolution and healing of the ulcers:

Experiment 1.—*Macacus rhesus* (weight, 2600 grms.) was injected with 9 c.c. gastrotoxic serum. It died on the *third day.* There was a patch of necrotic tissue and hæmorrhage, 1 inch by ¾ inch in size, in the wall of the stomach at the site of injection which was about to separate, forming an ulcer.

Experiment 2.—Bonnet Monkey (weight, 1530 grms.) was injected with 7 c.c. gastrotoxic serum and killed on the *fifth day.* An ulcer the size of a shilling with a slough covering the base and separated from the edges was found at the site of injection. There was some lymph on the outside of the stomach fixing it to the liver. The ulcer extended through the muscular coat of the stomach.

Experiment 3.—*Macacus rhesus* (weight, 2230 grms.) was injected with 7 c.c. gastrotoxic serum. It died on the *sixth day.* An ulcer was present in the stomach the same size as that of Experiment 2. The edges and base were clean, and there was a large perforation in the latter. There was recent lymph round the region of the ulcer and some free fluid in the abdomen.

Experiment 4.—Common Macaque (weight, 2630 grms.) was injected with 12 c.c. gastrotoxic serum and killed on the *thirteenth day*. An ulcer the size of a sixpenny-piece was present in the stomach. Its base was adherent to the liver. The edges were clean, rounded and healed, but the base itself was uncovered by epithelium, which was growing inwards from the periphery.

Experiment 5.—*Macacus rhesus* (weight, 3950 grms.) was injected with 10 c.c. gastrotoxic serum. It was killed on the *twenty-first day* following the injection. A scar was found in the stomach. It consisted of a small depressed surface surrounded by considerable puckering of the mucous membrane. On *microscopical examination* the depressed surface of the scar was seen to be completely covered with a single layer of epithelial cells (Plate XVI. Fig. 1).

Experiment 6.—*Patas cercopithecus* (weight, 2130 grms.) was injected with 12 c.c. gastrotoxic serum. The animal was killed on the *twenty-seventh day*. A healed ulcer was present in the stomach. The scar was stellate in form.

Experiment 7.—*Macacus rhesus* (weight, 2350 grms.) was injected with 9 c.c. gastrotoxic serum. At the same time the wall of the stomach around the site of injection was stitched to the liver, to prevent contraction of the base of the ulcer. The animal was given a bismuth meal, and the stomach found to empty itself in the normal time. It was killed on the *twenty-first day* following the injection. An ulcer was found in the stomach about the size of a threepenny-piece. The edges were smooth and rounded, and on *microscopical examination* were found to be covered with epithelial cells which had grown for a certain distance on to the base of the ulcer. An area in the centre of the base consisted of granulation tissue and was still uncovered by epithelium. There was no necrosis of the surface of the granulation tissue.

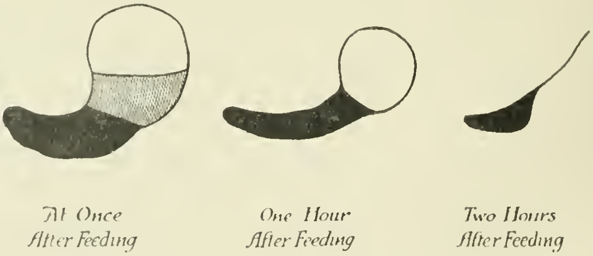


Fig. 1.—Tracings of the X-ray appearances of the stomach of Experiment 7 during the process of emptying, which occurred normally. The upper unshaded portion represents an air space. The less dense portion of the shadow is due to settling of the bismuth.

When, therefore, the stomach empties itself in the normal time, and the acidity of the gastric contents is normal, the ulcer may be expected to heal in three weeks or shortly after that time; but when the edges are prevented from coming together the healing occurs rather more slowly, because there is a larger area to be covered by epithelium. In the latter case the uncovered area is coated with granulation tissue as in the normal healing of the ulcer.

Production of hyperchlorhydria.—In a former series of experiments in which guinea-pigs were used, hyperchlorhydria was produced by feeding the animals upon food soaked in 0·5 or 0·6 per cent. HCl solution (1910 [7]). On estimating the stomach contents it was found that the acidity varied considerably, sometimes being normal and sometimes a little above normal, namely, 0·25 to 0·3 per cent. There was no delay in the healing of these ulcers, but I did not regard the method as being satisfactory, because one could not test the effect of any given strength of acid, and further one had no guarantee what was the percentage of *free* HCl in any given case, as the food converts the free HCl into protein HCl. The damage to the stomach wall is done by free HCl, not protein HCl. In this series of experiments, therefore, a solution of HCl was introduced into the fasting stomach. This is quite an easy procedure. A wooden gag with a hole in the centre was introduced into the monkey's mouth and a rubber tube passed through it into the stomach.

In the earlier experiments the animals were given the solution once a day in the morning, one hour before being fed. In the later experiments this was done before the evening meal also. In most of the cases the amount introduced on each occasion was 50 c.c., smaller amounts occasionally being used, particularly in the earlier experiments. Various strengths of HCl were used.

In hyperchlorhydria in the human subject the total hydrochloric acid secreted varies in different cases from 0·25 to over 0·4 per cent. At the end of two hours after a test meal of meat, the percentage of free HCl in the stomach contents is more than half that of the total amount of acid secreted in about 50 per cent. of the cases, and in the rest it is somewhat less than this. This statement is based on a series of actual estimates. The proportion of free HCl to protein HCl increases till the stomach is empty, and any damage to the stomach wall is therefore done in the later stages of digestion and is proportional to the length of stay of the residual contents in the stomach. In the case of associated hypersecretion the damage done is much greater, because the gastric mucous membrane is in this case exposed to the full strength of the HCl of the gastric juice for a longer time and in the fasting stomach.

The *efficiency* of this method of producing hyperchlorhydria is not great, and the effects obtained by it must be small in comparison with those produced by hyperchlorhydria in man. The reason for this is, that the solution rapidly leaves the stomach and the degree of acidity quickly falls. In illustration of this the three following experiments may be quoted:

Rhesus (weight, 2300 grms., Experiment 12) was given by the tube 50 c.c. of a 0·39 per cent. HCl solution. In half an hour 15 c.c. of fluid were found in the stomach containing 0·12 per cent. HCl.

Rhesus (weight, 3080 grms., Experiment 10) was given by tube 50 c.c. of a 0·28 per cent. HCl solution. In half an hour 28 c.c. of fluid were found in the stomach containing 0·14 per cent. HCl.

Rhesus (weight, 3150 grms., Experiment 8) was given by tube 50 c.c. of a 0·18 per cent. solution. In half an hour 7 c.c. fluid were found in the stomach containing 0·108 per cent. HCl.

The stomachs, therefore, in these experiments were exposed to the full strength of the solution administered for a short time only. This time was apparently shorter in the case of the stronger solutions than in that of the weaker ones.

The strengths of the solutions employed were 0·18, 0·28, 0·39, 0·5, 0·9, and 1·5 per cent. The solutions were made with concentrated HCl and distilled water, and the exact strength of each solution was afterwards ascertained by titration with decinormal soda solution. Solutions of 0·5 per cent. and upwards were liable to cause vomiting, owing to the irritant action of the HCl, and in such cases the maximum effect of the HCl was not obtained.

Effects of Hyperchlorhydria.

1. *Upon extension of the ulcer.*—In no case was any regular extension of the ulcer produced. When once the sloughs had separated, the epithelium was able to grow quite well if granulation tissue were present for it to cover.

It will be seen later that only HCl of a certain strength is able to kill the healthy gastric cells. In this case, when once the ulcer was formed, the gastric cells at the edge of the ulcer were healthy, and no regular extension of the ulcer occurred. The extension of an ulcer laterally is thus seen to be due to the persistence of the original cause of the ulcer, the gastric juice acting as a contributory factor only.

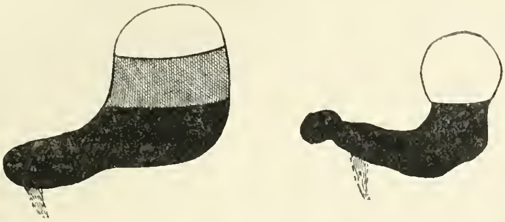
2. *Upon the healing of the ulcer.*—The effect of the hyperacidity of gastric juice was to produce a definite delay in the healing of the ulcer. This delay amounted to about three or four weeks beyond the normal time. The cause of the delay in the healing was the necrotic condition of the base of the ulcer. The hydrochloric acid, although unable to attack the epithelium at the edge of the ulcer, killed off the connective tissue of the base; the superficial layer of this for various depths was rendered necrotic; hence the growth of the epithelium over the base was retarded. In the case of normal healing the base of the ulcer, although uncovered by epithelium, is composed of granulation tissue after a certain period, but in unhealed ulcers on the twenty-first day in these cases of hyperacidity the granulation tissue was replaced by a slough owing to the necrotic action of the hydrochloric acid. When healing eventually occurred, the new mucous membrane was deformed, as has been described in a former paper when delayed healing was due to motor insufficiency of the stomach.

The following experiments were conducted in order to test the effect of various strengths of HCl.

Strength of HCl Solution = 0·18 *per cent.*

Experiment 8.—*Macacus rhesus* (weight, 3150 grms.) was injected with 7 c.c. gastrotoxic serum. On the fifth day feeding with HCl solution was commenced. For three days one feed was given each morning, and subsequently two feeds—one in the morning and one in the evening—were given daily with a very few exceptions. The amount administered was 50 c.c. on each occasion, and twenty-three feeds in all were given. An examination with the X-rays after a bismuth meal showed that the stomach emptied itself in the normal time. The monkey was killed on the twenty-first day, and an unhealed ulcer, triangular in shape, was found in the stomach, which was adherent to the liver. The edges of the ulcer were covered with epithelium, and this was growing over the base, but the centre of the latter was covered with sloughy material preventing the further growth of the epithelium. The base was considerably contracted.

Experiment 9.—*Macacus rhesus* (weight, 2550 grms.) was injected with 6 c.c. gastrotoxic serum. On the sixth and seventh days the monkey was fed with 50 c.c. of HCl solution, and on the eighth day feeding twice a day was commenced as in the preceding experiment. Seventy-eight feeds were given. The stomach was found by the X-rays to empty itself in the normal time. The monkey was killed at the end of nine weeks and three days. The stomach was adherent to the liver and contained an excess of mucus. A radiating scar was present in the mucous membrane at the adherent spot.

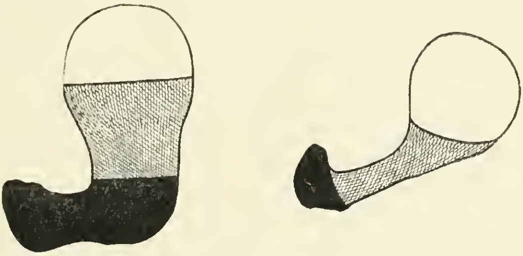


Twenty Five Minutes After Feeding *Two Hours after feeding Stomach empty in Three and a Half Hours*

Fig. 2.—Tracings of the X-ray appearances of the stomach of Experiment 9 during the process of emptying, which occurred normally.

Strength of HCl Solution = 0·28 *per cent.*

Experiment 10.—*Macacus rhesus* (weight, 3080 grms.) was injected with 7 c.c. gastrotoxic serum. Feeding with HCl solution was commenced on the fifth day after injection. On the first three days one feed a day was given, and after this period the monkey was, with one or two exceptions, fed twice a day. The volume of the feed on each occasion was 50 c.c. Twenty-three feeds were administered. The stomach was found by the X-rays to empty itself in the normal time. The monkey was killed on the twenty-first day. An unhealed ulcer, ¼ inch in size, was present in the stomach; its base was considerably contracted and in a similar condition to that of Experiment 8.



Half an Hour After Feeding *Two and a Half Hours After Feeding*

Fig. 3.—Tracings of the X-ray appearances of the stomach of Experiment 10 during the process of emptying, which occurred normally.

Experiment 11.—*Macacus rhesus* (weight, 3100 grms.) was injected with 7 c.c. gastrotoxic serum. Feeding was commenced on the sixth day after injection. On the first day one feed was given, and subsequently two each day. The amount of each feed was 50 c.c. Sixty-seven feeds were administered. The stomach was proved by the X-rays to empty itself in the normal time. The monkey was killed at the end of seven weeks. A scar was found in the mucous membrane of the stomach, 1 inch by $\frac{5}{8}$ inch in size. The edges were raised, and the base which was covered with newly-formed mucous membrane sloped slightly to the centre. The ulcer was completely healed.

Strength of HCl solution = 0·39 *per cent.*

Experiment 12.—*Macacus rhesus* (weight, 2300 grms.) was injected with 8 c.c. gastrotoxic serum. Feeding with HCl solution was commenced on the fifth day. On the first three occasions one feed only was given during the day, and subsequently the animal was fed twice a day. The amount of the feeds was 50 c.c. Twenty-three feeds were administered. The stomach emptied normally. The monkey was killed on the twenty-first day. An excess of bile-stained mucus was present in the stomach. An ulcer was found with healed edges and contracting base, over which the epithelium was growing, the centre being covered with slough. The ulcer was larger than those of Experiments 8 and 10 (Plate XVI. Fig. 2).

Experiment 13.—*Macacus rhesus* (weight, 2550 grms.) was injected with 8 c.c. gastrotoxic serum. Feeding was commenced on the fifth day. In this case only one feed a day was given, the total number of feeds being seventeen. The animal was killed at the end of a month. An unhealed ulcer, lozenge-shaped and $\frac{3}{4}$ inch by $\frac{3}{8}$ inch in size, was present in the stomach. It had raised edges and the base sloped to the centre in the shape of a funnel. The whole was covered with newly-formed mucous membrane, except a slit-shaped portion in the centre where a slough was present.

Experiment 14.—*Macacus rhesus* (weight, 2800 grms.) was injected with 6 c.c. gastrotoxic serum Feeding with 50 c.c. of HCl solution was commenced on the fifth day. For four days the feeds were administered once a day, and subsequently twice a day. One hundred and twelve feeds were administered. By X-rays the stomach was found to empty normally. The monkey was killed at the end of eleven weeks. The stomach was adherent to the liver, and there was very slight hour-glass constriction at the spot. The stomach contained a good deal of mucus. A scar was present, which was 1 inch by $\frac{1}{2}$ inch in size. The edges were raised and the base of the scar covered with newly formed mucous membrane. In the centre was a puckered knot. An hour before it was killed the animal was given 50 c.c. of milk as a test meal. The total HCl secreted was found, on analysis of the stomach contents, to be 0·057 per cent.

Strength of the HCl solution = 0·5 *per cent.*

Experiment 15.—*Macacus rhesus* (weight, 2830 grms.) was injected with 7 c.c. gastrotoxic serum. Feeding with 50 c.c. of the solution was commenced on the fourth day, and after the first three feeds was continued twice a day. Twenty-six feeds were administered. Vomiting occurred on several occasions. The stomach was shown to empty itself in the normal time. The monkey was killed on the twenty-third day. A healing ulcer was found in the stomach. It was well-contracted, deep, and the uncovered portion of the base was about the size of a split pea. A good deal of mucus was present in the stomach.

Experiment 16.—*Macacus rhesus* (weight, 2900 grms.) was injected with 7 c.c. gastrotoxic serum. Feeding with 50 c.c. HCl solution was commenced on the fourth day and continued twice a day after the first three doses. Vomiting occurred off and on, and was accompanied by salivation and a good deal of retching. Thirty-seven feeds were administered. The stomach emptied in the normal time. The monkey was killed on the thirtieth day. There was a good deal of mucus in the stomach and a small deep ulcer was present. It was healing slowly, oval in shape, and $\frac{3}{8}$ inch by $\frac{1}{4}$ inch in size.

Experiment 17.—*Macacus rhesus* (weight, 2260 grms.) was injected with 9 c.c. gastrotoxic serum. Feeding with 50 c.c. HCl solution was commenced on the seventh day after injection. The feeds were given once a day only, and twenty-nine in all were administered. Vomiting occurred on several occasions. By the X-rays the stomach was found to empty itself in the normal time. The monkey was killed on the fortieth day. An ulcer advanced in healing was present in the stomach. It was 1 inch by ½ inch in size. It was funnel-shaped, with raised edges. The newly-formed mucous membrane had grown down the sides, leaving a small slit-shaped area in the centre of the base covered with slough.

Strength of HCl Solution = 0·9 *per cent.*

Experiment 18.—Baboon (weight, 1350 grms.).—Injected with 8 c.c. gastrotoxic serum. Feeding with HCl solution was commenced on the eighth day. The feeds were given once a day; for the first four days 30 c.c., and for the next seven 40 c.c., were given on each occasion. The monkey vomited every day. It was killed on the twenty-first day, and a healed ulcer was found in the stomach. The scar was stellate in form.

Strength of HCl Solution = 1·6 *per cent*

Experiment 19.—*Macacus rhesus* (weight, 2370 grms.) was injected with 8 c.c. gastrotoxic serum. Feeding was commenced on the seventh day and continued daily. Twelve doses of 20 c.c. and three of 30 c.c. were administered. The animal vomited and suffered from salivation. It was killed on the twenty-fourth day. An ulcer was present in the stomach. It was healed except for a tiny area in the centre. The stomach wall was puckered, but there were no adhesions.

Free hydrochloric acid of all the ordinary strengths found in hyperacidity of the gastric juice, in human subjects suffering from pathological conditions, was able to delay materially the healing of the ulcer, but in no case was complete arrest of healing produced. This may of course have been due to the inefficiency of the method employed, and it is quite certain that hyperchlorhydria as found in the human subject would produce a much greater effect. A solution of 0·18 per cent. strength was able to produce *some* effect. Solutions of 0·5 per cent. and upwards did not appear to produce more effect than the weaker solutions, and sometimes much less. This was due to the fact that they gave rise to vomiting in the first place; moreover, a stronger solution in the short time at its disposal can do little more than kill off the superficial granulation tissue in the same way as a weaker solution. The delay in healing in all cases was due to the same cause, namely, necrosis of the granulation tissue base of the ulcer, which prevented the epithelium growing over it. Gastric juice is the only digestive fluid which is able to digest connective tissue, and therefore disintegrate food-stuffs and organs, whose elements are bound together by that tissue. This power it owes to the hydrochloric acid contained in it. It is not surprising, therefore, that hyperacid gastric juice should attack the connective-tissue base of an ulcer and produce the results described above.

Another method was employed to test the effect of the secretion of gastric juice in the fasting stomach upon the healing of gastric ulcer.

It is well known that *pilocarpine* on injection causes a flow of gastric juice, but I could not find in the literature on the subject whether, while the flow continues, the juice contains the normal amount of acid, and whether repeated injection of pilocarpine alters the acid content of the juice. A few experiments on cats were therefore conducted in order to determine these points.

METHOD.—The cat, which had been starved for twenty-four hours previously, was anæsthetised with ether. The abdomen was opened in the middle line and the stomach drawn out. Ligatures were placed round the lower end of the œsophagus and the duodenum just beyond the pylorus. An incision was made into the stomach, and any remnants of mucus carefully removed by mopping out with absorbent cotton wool. The edges of the incision were stitched to the edges of the abdominal wound so as to make a gastric fistula with a large external opening. The pilocarpine was injected subcutaneously or intravenously and the gastric juice collected by means of a pipette at intervals.

Experiment A.—CAT (weight, 2750 grms.).—Fistula prepared as described above, and pilocarpine nitrite, ⅓ gr., injected into the femoral vein at 10.25 a.m. Salivation commenced in a few minutes, and the following amounts of gastric juice were collected :

At 11.45 a.m.	2·5 c.c.	
12.45 p.m.	1·5 ,,	
1.45 ,,	1·5 ,,	
2.45 ,,	2 ,,	
3.45 ,,	1·5 ,,	killed.

The juice was yellowish in colour, with a few tiny brownish flakes, evidently due to minute hæmorrhages from the gastric mucous membrane. The salivation diminished considerably after the first hour. The samples of juice were mixed together and the amount of HCl estimated by the silver method. The total chlorides were found to amount to 0·1898 per cent. The total acidity was then estimated with decinormal NaOH and found to amount to 0·1825 per cent. The chloride is therefore present almost entirely as free HCl.

Experiment B.—CAT (weight, 2210 grms.).—Fistula prepared. Pilocarpine nitrate, ⅓ gr., subcutaneously injected at 11.45 a.m. In about three minutes salivation commenced, and at the same time the gastric mucous membrane became moist. At 12.45 p.m. (one hour later) 8·5 c.c. of gastric juice had been collected when the animal died. On estimation the percentage of HCl in the juice was found to be 0·18 per cent.

Experiment C.—CAT (weight, 3390 grms.).—Fistula prepared. Pilocarpine nitrate, ⅙ gr., subcutaneously injected at 10.50 a.m. At 12 p.m. 6 c.c. gastric juice containing 0·18 per cent. HCl, and at 1 p.m. 3½ c.c. juice containing 0·146 per cent. HCl, were collected. The animal was then killed.

Experiment D.—CAT (weight, 2800 grms.).—Fistula prepared. Pilocarpine nitrate, 1/12 gr., subcutaneously injected at 10.25 a.m. The following samples of gastric juice were collected.

Time.	Salivation.	Gastric Juice.	HCl.
11.30 a.m.	Profuse	2 c.c.	0·18 per cent.
1.30 p.m.	Some	3 c.c.	
3.30 p.m.	Very slight	3·5 c.c.	0·146 ,,
5.30 p.m.	None	2 c.c.	0·109 ,,
Animal killed	...	...	...

Experiment E.—Cat (weight, 3000 grms.) was injected repeatedly with pilocarpine nitrate. Twelve injections were given in thirty-two days. Four doses of $\frac{1}{6}$ gr., seven doses of $\frac{1}{3}$ gr., and one dose of $\frac{1}{2}$ gr. were administered. Two days after the last injection the experiment was performed. A gastric fistula as described above was made, and pilocarpine nitrate, $\frac{1}{3}$ gr., subcutaneously injected. Six c.c. gastric juice containing 0·18 per cent. HCl was collected in three hours, when the animal was killed.

The amount of gastric juice secreted in response to the injection of a given dose of pilocarpine varies somewhat in quantity. The percentage of HCl present in it is constant, but it tends to diminish a little after the first hour of secretion. Repeated injections of the drug do not affect the quantity and quality of the gastric juice secreted as the result of its action. Injections of pilocarpine, therefore, may be used to test the effects of gastric juice, unadulterated with food, upon the healing of an ulcer.

With this end in view pilocarpine was repeatedly injected into a monkey, in which an ulcer had been produced.

Experiment 20.—*Macacus rhesus* (weight, 3200 grms.) was injected with 9 c.c. gastrotoxic serum. The monkey received twenty-two subcutaneous injections of pilocarpine, not more than one a day being given. The doses were as follows: $\frac{1}{6}$ gr. was injected twice, $\frac{1}{3}$ gr. eight times, $\frac{1}{2}$ gr. seven times, and $\frac{2}{3}$ gr. five times. The injections were given in the morning two hours before the first feed. The animal was killed at the end of six weeks. What appeared to the naked eye to be a linear scar, 1 inch by $\frac{1}{16}$ inch in size, with puckering of the mucous membrane on both sides of it, was present in the stomach. On *microscopic examination*, however, it was seen that the linear space was uncovered by mucous membrane, and therefore the ulcer was not yet healed. The newly formed glands were often found distended into mucous cysts.

Hypersecretion of normal gastric juice into the fasting stomach, therefore, is able to delay to some extent the healing of the ulcer. This experiment confirms the results of those recorded above, in which a 0·18 per cent. HCl solution was found to produce the same effect.

II.

EFFECTS OF HYPERCHLORHYDRIA, TOGETHER WITH MOTOR INSUFFICIENCY, UPON THE HEALING OF GASTRIC ULCER.

In a former paper it was shown that motor insufficiency, leading to retention of food in the stomach, definitely delayed the healing of acute gastric ulcer (1910[3]); and that this effect was due to necrosis of the granulation tissue base of the ulcer, the growth of the epithelium over the latter being thus delayed. The prolonged action of HCl was responsible for this result. These experiments were performed upon cats. Retention of food in the stomach, for varying lengths of time, is present, as stated above, at one time or another in all cases of chronic gastric ulcer. It seemed, therefore, natural to inquire how much further delay could be produced by motor insufficiency acting in conjunction with hyperacidity.

In this series of experiments also, monkeys were used. The ulcers were produced by the method described above, and the animals were fed with solutions of HCl by the tube.

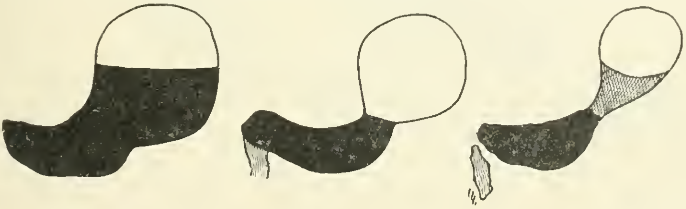
Production of motor insufficiency.—The motor insufficiency was produced by artificial duodenal obstruction. The method of obstruction of the first part of the duodenum by a piece of indiarubber tubing, which I had found to be successful in the cat, was inapplicable to the monkey. This was owing to the position of the stomach and the connections of the duodenum. The duodenum of the cat is more movable and the stomach is not slung from it as is the case in the monkey, in which animal I found that kinking resulted and complete obstruction was produced. Neither was the method of introducing a row of Lembert's sutures very successful, on account of the difficulty of judging of the degree of constriction produced, and in such a condition compensation was easily produced. The method which I finally adopted was the following.

A median incision was made in the abdomen, with strictly antiseptic precautions under ether. The stomach was drawn out and a very small gastroenterostomy clamp put on the organ so as to separate off the body, into which the contents of the stomach were squeezed, from the pyloric region. A small incision was made into the latter and a glass rod about 4 mm. in diameter was passed into the opening and through the pylorus into the duodenum. Two pieces of silver wire were passed round the first part of the duodenum immediately beyond the pylorus, and each separately twisted on the glass rod, which was firmly held in position. The degree of tightness secured was one which admitted of the glass rod being withdrawn and reintroduced fairly easily through the stenosed duodenum. The glass rod was then taken out and the incision into the stomach closed by silk sutures. The gastrotoxic serum was injected into the stomach wall, as described above, at the same operation. The animal had been given a small meal one hour before the operation.

The result of the obstruction was to produce some loss of weight and diminished appetite, and in the higher degrees a certain amount of vomiting. The animals, however, kept well in all other respects.

Estimation of the presence and the degree of retention of food.—The animals were not all of the same size and the degree of tightness of the wires varied a little, so that it was necessary to estimate the actual degree of obstruction in each case, in order to compare this with the results obtained. The method used was that of watching by the X-rays the rate of emptying of the stomach after having administered a bismuth meal. The meal was administered by the stomach tube and consisted of 1 drm. of bismuth carbonate suspended in 30 c.c. of milk. The bismuth settles a little in the stomach, but when the milk clots it becomes mixed with the clot and the outline of the stomach is very clearly and definitely shown.

Normal position, shape, and emptying of the stomach.—The outline of the stomach was drawn on the screen at intervals and then traced on oiled paper. The exact position was easily located by drawing in the diaphragm and outlines of the body wall. This was found to be much the simplest method. Radiograms were taken in some instances. Unless taken as snapshots they were blurred, owing to the respiratory excursions, and possessed no advantage over the diagrams. The monkeys were all examined in the upright position. The shadow of the stomach consists of a vertical portion on the left of the mid-line and a shorter and narrower horizontal portion which turns to the right. There is, as in man, a fair-sized air space in the fundus. The appearance presented is practically the same as that of a baby a few weeks old. The upper part of the vertical shadow for a variable depth is usually less dense, owing to settling of the bismuth. When water instead of milk is given the bismuth settles rapidly, forming a layer in the most dependent portion of the stomach. After the milk has clotted, the bismuth is mixed with the clots and the fluid portion is uncoloured. The fluid part leaves the stomach more rapidly than the clots. The shadow becomes narrower and smaller, and finally a small black mass occupying the distal end of the pyloric region is all that can be seen; eventually this disappears. A certain amount of narrowing is quite common at the junction of the pyloric region and the body, forming various degrees of spasmodic hour-glass stomach. The movements of the stomach, as seen at the moment by the X-rays, are very sluggish, probably owing to fright. This may not be the case, because I have seen the same sort of movements in a baby, which was not frightened. The stomach commences to empty at once, and after a quarter of an hour or thereabouts bismuth may be seen in the small intestine, the coils of which become rapidly outlined as the stomach empties (Fig. 4). After a meal of 30 c.c. milk and 1 drm. of bismuth carbonate, the stomach is usually empty in from two and a half to three and a half hours, according to the size of the monkey. The larger the meal the longer the stomach takes to empty; for example, after a meal of 50 c.c. it is not empty within about five hours.



Quarter of an Hour After Feeding *One and a Quarter Hours After Feeding* *Two and a Quarter Hours After Feeding*

FIG. 4.—Tracings of the X-ray appearances of the stomach of a normal monkey during the process of emptying. The stomach was empty within four hours. The air space was fairly large and the stomach showed spasmodic hour-glass contraction. The bismuth meal could be seen passing out of the pylorus.

Rate of emptying in the condition of pyloric stenosis.—The rate of emptying was found to vary with the degree of obstruction. The silver wires round the duodenum could be quite easily seen with the X-rays. In the lesser degrees of obstruction the size and shape of the stomach was normal, in the higher degrees the stomach was dilated. In addition to the size of the stomach, two other points were obvious. The first was that as the food collected in the small intestine much more slowly than normal, the shadow in that organ appeared more slowly than usual, and with higher degrees of obstruction the bismuth could not be seen till it reached the large intestine. The second was, that the size of the bismuth shadow in the stomach diminished more slowly than normal and did not disappear until a varying number of hours beyond the normal time limit.

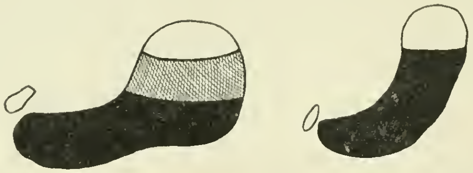
Having decided upon the methods to be employed, the following experiments were performed:

Strength of HCl Solution = 0·28 per cent.

Experiment 21.—*Macacus rhesus* (weight, 2080 grms.) was injected with 7 c.c. gastrotoxic serum, and at the same operation the duodenum was stenosed with silver wire, as described above. Feeding with the HCl solution was commenced on the fifth day after injection, 50 c.c. being administered on each occasion. A single feed was given on the fifth, seventh, and eighth days respectively, after which the animal was, with four exceptions, fed twice a day.

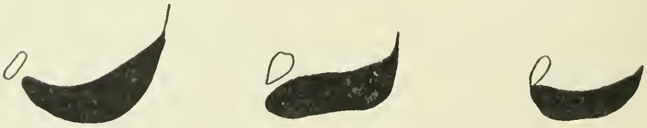
Twenty-one feeds in all were administered. On the fourteenth day a bismuth meal was given. The stomach emptied slowly, and after six and a quarter hours still contained a certain amount of food. The animal was killed on the twenty-first day. It was given 50 c.c. 0·28 per cent. HCl solution in the morning and killed in half an hour. The stomach contained 30 c.c. of a clear, slightly mucoid fluid, containing 0·19 per cent. free HCl.

There was a large ulcer in the stomach, 1 inch by $\frac{5}{8}$ inch in size, adherent to the liver and a little excavated in the centre; the base was covered with a yellowish slough. The edges were rounded and the mucous membrane beginning to grow over the periphery of the base (Plate XVI. Fig. 3).



Half-an-Hour After Feeding

Two Hours and Fifty Minutes After Feeding



Four and a Quarter Hours After Feeding

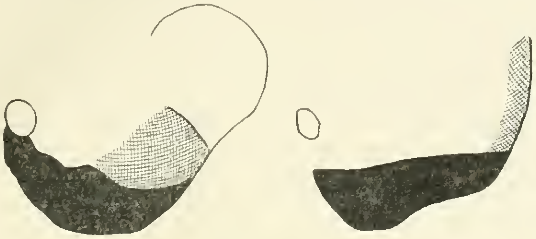
Five and a Quarter Hours After Feeding

Six and a Quarter Hours After Feeding

Fig. 5.—Tracings of the X-ray appearances of the stomach of Experiment 21 during the process of emptying. The silver ring round the duodenum is distinctly seen. There is considerable delay in the emptying, a large amount of food being present in the stomach after six and a quarter hours. There is no dilatation of the stomach.

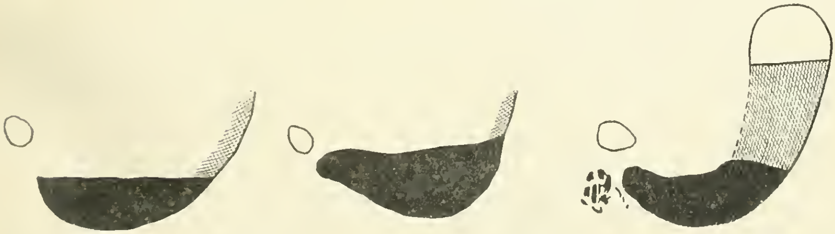
Strength of HCl Solution = 0·39 per cent.

Experiment 22.—Bonnet Monkey (weight, 2180 grms.) was injected with 8 c.c. gastrotoxic serum, and at the same operation the duodenum was obstructed. One feed of 50 c.c. HCl solution was administered on the fourth, fifth, sixth, and eighth days respectively after injection. After this period the feeds were given twice a day. Twenty-five feeds in all were administered. A bismuth meal was given on the seventh day. It was administered in the morning, all food having been taken away from the animal at 6 p.m. on the previous evening. From the shape of the shadow it appeared that a certain amount of residual food had been retained all night. The stomach emptied slowly, and eight hours after administration still contained a considerable amount of food. The animal was killed on the twenty-first day after injection of the serum. A large ulcer, 1 inch by $\frac{1}{2}$ inch in size, was found in the stomach, which was adherent to the liver. The base of the ulcer was completely covered with slough. The edges were rounded and the mucous membrane was just beginning to grow over the periphery of the base (Plate XVI. Fig. 4).



Ten Minutes After Feeding

One Hour After Feeding



Three Hours After Feeding

Five Hours After Feeding

Eight Hours After Feeding

FIG. 6.—Tracings of the X-ray appearances of the stomach of Experiment 22 during the process of emptying. The silver ring round the duodenum is seen. There is some dilatation of the stomach and considerable delay in the emptying, a large amount of food being retained after eight hours.

Experiment 23.—BONNET MONKEY (weight, 1580 grms.) was injected with 8 c.c. gastrotoxic serum, and at the same operation the duodenum was narrowed by silk sutures. Feeding with HCl solution was commenced on the fifth day, and continued once a day only, as the animal, although bright looking, was obviously losing ground. Five doses of 10 c.c. each were given, and then one dose of 20 c.c., and three doses of 30 c.c. The animal died on the sixteenth day. The duodenal constriction was tight. The stomach was adherent to the liver, spleen, and diaphragm. There was a large ulcer at the site of injection, with inverted edges and an excavated cavity. It was 1 inch by $\frac{3}{4}$ inch in size. Microscopic sections showed that the base was covered with slough and that the epithelium of the edge had not commenced to grow over it. A second and newly-formed ulcer was present on the posterior wall, parallel to and quite near the small curvature. It was oval in shape, longitudinally situated, $\frac{3}{4}$ inch by $\frac{1}{4}$ inch in size. It had sharply-cut edges, and the muscular coat of the stomach was exposed in the base. There were no signs of healing, and the edge showed disappearance of the gland such as occurs in acute spreading ulcer in man. One or two tiny ulcers were forming in the immediate vicinity (Plate XVII. Fig. 5). Sections of the small commencing ulcers showed that the process of ulceration commenced as a necrotic lesion in the mucous membrane. This is a very important experiment, as it illustrates the commencement of an acute spreading ulcer in necrosis of the mucous membrane due to HCl. This point will be reverted to later.

Strength of HCl Solution = 0·9 *per cent.*

Experiment 24.—*Macacus rhesus* (weight, 2830 grms.) was injected with 10 c.c. gastrotoxic serum. At the same operation the duodenum was constricted with silver wire. A bismuth meal was given on the seventh day. The meal consisted of 50 c.c. milk and 1 drm. of bismuth carbonate. At the end of eight and a half hours there was a large mass of food present in the stomach. Feeding with the HCl solution was started on the eighth day, one feed a day being administered. Four doses of 30 c.c. each and seven doses of 40 c.c. each were given in all. The monkey was killed on the twenty-first day. A large ulcer with inverted edges was present in the stomach and was adherent to the liver. It was $\frac{3}{4}$ inch by $\frac{5}{8}$ inch in size. The base was white and sloughy and completely uncovered (Plate XVII. Fig. 6). On section it was found that the epithelium of the edge was just beginning to grow on to the periphery of the base. Two or three small ulcers were also present in distant parts of the stomach.

In all these experiments the ulcers were larger and less advanced in healing than in the former series of experiments in which hyperacidity alone was produced. The cause of the delay in healing was the same, namely, necrosis of the base of the ulcer, so that the mucous membrane was unable to reform. The edges of the ulcers were rounded, and there was no tendency for the latter to spread.

There was here a summation of effects, each factor—hyperacidity and food retention—contributing to delay the healing of the ulcers. It is probable that the ulcers would have eventually healed, although one cannot be quite certain on this point, as no case was examined beyond the twenty-first day.

It is quite certain that hyperacidity and motor insufficiency in man are able to produce greater effects than those described in these experiments.

It is to be noted that there were no appearances in the X-ray pictures to suggest that an ulcer was present in the stomach. This could hardly be expected to occur unless the ulcer formed a pouch on one or other curvature so as to interrupt the contour of the shadow. The ulcers in this position had no effect whatever upon the movements of the stomach.

In the case of a small baboon an hour-glass contraction of the stomach was produced. This could be seen on the X-ray screen, since the only part of the stomach behind the stricture contained food, leaving the pyloric portion empty. After a while, however, the pyloric portion filled up, the former shadow diminishing in size, and it was then quite impossible to recognise the contraction at this stage.

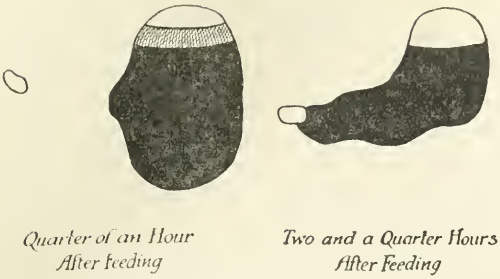


FIG. 7.—Tracings of the X-ray appearances of the stomach of the baboon mentioned above. The silver ring round the duodenum is seen. In the first tracing the pyloric region is quite empty, but in the second the outline of the stomach is normal. The organic hour-glass contraction had obviously been so increased by spasm as to prevent the passage of food.

The stomach contents of those animals which died from the tightness of the stricture showed a diminished amount of HCl, exact figures obtained in three cases being 0·018, 0·036, and 0·072 per cent. acid. In the lesser degrees of obstruction the acidity was unaffected, being from 0·1 to 0·2 per cent.

III.

EFFECTS OF HYPERCHLORHYDRIA UPON THE GASTRIC MUCOUS MEMBRANE.

It was found that the solutions of HCl produced certain well-marked changes in the gastric mucous membrane. These changes were worked out in the animals whose records have already been described and also in certain additional animals. A general description will be given of these changes, as it is unnecessary to refer in detail to each animal investigated. The effects of the repeated doses of HCl solutions upon the gastric mucous membrane were irritant and necrotic.

1. IRRITANT EFFECT.—This effect was manifested as a chronic inflammation of the mucous membrane. On opening the stomach an increased amount of mucus was found in it. Points of hæmorrhage were to be seen in the mucous membrane in the advanced cases, but not in the slighter cases. The mucous membrane itself was not particularly congested, but, on the contrary, was very often paler than normal. Enlarged lymph follicles could be seen under the mucous membrane scattered about the stomach like seeds, and in more advanced cases they opened on the surface by small pinholes constituting follicular ulcers (Plate XVII. Fig. 7). Another appearance presented was that of rounded patches slightly depressed below the surface of the mucous membrane and surrounded by a well-defined

rim of normal tissue. They varied in size up to about a quarter of an inch in diameter. These proved to be localised patches of gastritis (Plate XVII. Fig. 8). In no case was there any evidence that the follicular ulcers had spread by continued administration of the acid so as to form larger ulcers.

Microscopical examination of the stomach showed that the gastritis was either a more or less diffuse condition or that it was localised to patches surrounded by perfectly healthy mucous membrane. The *gastritis*, whether it was diffuse or localised, gave rise to the same appearances in all cases.

The most apparent change was the *infiltration with round cells* of the mucous membrane affected. This infiltration which existed between the glands was present in varying degrees according to the intensity of the disease. It was most marked towards the surface of the mucous membrane and less towards the bases of the glands, in the slight cases merely packing the interglandular tissue, in the more advanced cases compressing and invading the glands, which became less defined and gradually disappeared (Plate XVIII. Fig. 9).

The *surface epithelium* in early cases showed swelling with mucus, and there was often a deposit of mucus on the surface of the membrane. In the more advanced cases desquamation of the epithelium occurred, and on the surface was a layer of mucus with loose cells shrunken, deformed, and entangled in it.

In these cases the *cells of the glands* were in a similar condition to those of the surface epithelium. In advanced cases the glands had largely disappeared, and those which were left appeared as islands of cells, sometimes still arranged around the central lumen or sometimes desquamated into it and embedded in the infiltrated tissue.

There was no doubt whatever that the desquamated surface epithelial cells were renewed by the proliferation of unaffected cells, because young flattened cells could be seen on the surface in many sections. These newly-formed surface cells were no doubt desquamated in their turn. In this way the process was continued, any uncovered surfaces which might be said to constitute "erosions" being rapidly covered over, so that open ulcers were not present for very long. The *underlying tissue* consisted of small round cells and proliferated connective-tissue cells with the remains of the glands embedded in it.

The *lymphatic follicles* in the normal condition are rounded compact masses of cells situated under the muscularis mucosæ. They may also be embedded in the mucous membrane and there lie on the muscularis mucosæ, having in section a somewhat triangular shape owing to their extension upwards between the bases of the glands. In either case they become enlarged and spread upwards to the surface of the mucous membrane and also laterally. Those lying under the muscularis mucosæ burst first through that structure. By proliferation of their cells the nodules thus grow upwards, displacing and pushing aside the gastric glands, many of which are destroyed in the process. Eventually the nodule reaches the surface and bursts through the epithelium, producing a tiny acute ulcer (Plate XVIII. Fig. 10). In no case was degeneration of the cells forming the nodule observed, leading to the condition of abscess formation. The lymph cells always stained well even after the surface epithelium was destroyed.

Follicular ulcers of various sizes were thus produced, and the same process of repair as is described above was observed here. Many ulcers were found, but in many cases also the surface of the ulcer was already covered with newly-formed epithelium which had grown in from the edges of the ulcer. Extension of the ulcer either laterally or in its depth was never observed. These follicular ulcers might be found quite isolated in the midst of almost normal mucous membrane. On the other hand, their occasional presence in connexion with a local patch of gastritis gave rise to the suggestion that the patch might have arisen as an extension from the enlarged follicle. Many patches of gastritis were, however, found quite free from enlarged follicles.

This irritant effect of hydrochloric acid was produced with solutions of 0·28 per cent. in strength, and of course with any stronger solution. I have already stated that solutions of 0·5 per cent. strength and over often caused vomiting, so that the effects upon the mucous membrane varied a little, but, speaking generally, the degree and extent of the gastritis varied in proportion to the strength of the acid and the length of time over which it was administered. Quite a marked effect, however, was seen in the stomach of animals who had only received a few doses.

The regularity with which this gastritis is produced by weak solutions of HCl acting for such short periods makes it quite certain that continued hyperchlorhydria in the human subject gives rise to gastritis.

Incidentally it may be mentioned that the presence of gastritis did not affect the healing of the ulcers. This agrees with the observations of pathological anatomy. Neither were the movements of the stomach affected.

2. NECROTIC EFFECT.—So far as we have seen the usual effect is an irritant one and there is merely a separation and killing off of surface epithelial cells, the glands being destroyed as a secondary effect of the inflammatory infiltration. Hydrochloric acid, however, is able in certain strengths to kill a tract of mucous membrane for various depths, and by continuous administration to produce a spreading ulcer.

I had previously found in the case of the guinea-pig that a single dose of a solution of HCl of 0·7 to 0·9 per cent. in strength, according to the weight of the animal, was able to produce patches of necrosis in the gastric mucous membrane (1910 [7]). Strengths below these were unable to do so unless the cells were also devitalised by some other cause. In the case of the monkey, stronger solutions than these are required. In the case of a bonnet monkey, weighing 1530 grms., 20 c.c. of a 1·6 per cent. HCl solution produced large patches of necrosis in the mucous membrane, stained black by altered blood.

I have found that weaker solutions than this, if continuously administered, are now and again able to produce necrosis, gradually extending in the mucous membrane and giving rise to a definite ulcer. The weakest solution which I have found to produce this effect in the monkey is one of 0·39 per cent. in strength.

Experiment 23 is an example of such a case. In this experiment an oval spreading ulcer was found, and one or two tiny ones in its vicinity. The animal had also marked duodenal obstruction. Another bonnet monkey, weighing 2800 grms., was injected with 8 c.c. gastrotoxic serum, and duodenal obstruction produced. On the fourth, fifth, and sixth days 50 c.c. HCl solution, 0·39 per cent. in strength, were administered. The animal wasted and looked ill, and was therefore killed on the seventh day. The constriction was very tight. An enormous ulcer occupying most of the anterior wall and small curvature had been produced at the site of injection. It was 2 inches by 1¾ inch in size, and exposed the liver. In addition, some necrotic ulcers quite apart from the injection were present. One was ¼ inch long, and there were two or three smaller ones. The enormous size of the ulcer, produced at the site of injection, was due to the action of the acid, aided, no doubt, by the obstruction; the presence of the smaller ulcers was entirely due to this (Plate XVIII. Fig. 12).

Another case illustrates the fact that in the condition of pyloric obstruction a gastrotoxic ulcer may be made enormous by the administration of 0·39 per cent. HCl solution in the early stages.

The monkey was a bonnet, and weighed 2230 grms. 8 c.c. gastrotoxic serum were injected and the duodenum obstructed. On the fourth, fifth, and sixth days 50 c.c. of 0·39 HCl solution were administered, and as the animal looked ill it was killed on the seventh day. The constriction was tight. A very large ulcer, 1½ inch by 1 inch in size, with hæmorrhage into the surrounding mucous membrane, was found. It had exposed the liver over a large area and the base was perforated at one edge. General peritonitis was commencing. There were no other ulcers to be seen by the naked eye.

Experiment 24, in which 0·9 per cent. HCl was administered, showed two or three small necrotic ulcers.

A 1 per cent. solution of HCl, repeatedly administered, is able to cause such ulcers in the cat, in the absence of pyloric obstruction.

Two cats, weighing 4420 grms. and 2755 grms. respectively, were fed with HCl solution. The former received two doses of 0·33 per cent. solution and three doses of 1 per cent. solution, each amounting to 50 c.c. It was then killed. There were several patches of superficial ulceration and a considerable number of scattered points of hæmorrhage in the stomach. The latter received sixteen doses of 60 c.c. of a 1 per cent. solution of HCl and was then killed. There was much mucus in the stomach, and a considerable amount of superficial ulceration of the mucous membrane (Plate XVIII. Fig. 11).

Necrosis of the mucous membrane of the stomach, therefore, only occurs with fairly strong solutions of HCl, provided that the stomach empties normally, but in the condition of pyloric obstruction it is liable to occur with solutions of 0·39 per cent. in strength.

Considering that such a short time is available for the action of these solutions, and that the human stomach is probably as susceptible to HCl solution as is that of the monkey or cat, it is probable that hyperacidity of, say, 0·35 to 0·4 per cent., and hypersecretion of the gastric juice, will now and again of themselves give rise to an ulcer of the stomach.

Sections of these necrotic ulcers were cut and the following points elicited. The ulcer begins as a tiny necrotic spot in the superficial mucous membrane, which separates. The necrosis spreads in all directions, and the tissue gradually separates until an ulcer involving the whole thickness of the mucous membrane is produced, quite comparable to the acute spreading ulcer in man. The smallest ulcers are circular and about as big as a pin's head. They often spread irregularly, but in the monkey, Experiment 23, the ulcer spread in the regular oval shape as described above, lying parallel to or near the small curvature on the posterior wall of the stomach.

CONCLUSIONS.

1. The gastric juice is not only concerned in the initiation of a gastric ulcer, but also in its tendency to spread and to become chronic. It is thus responsible for the anatomical peculiarities which distinguish this ulcer from ulcers in other parts of the body.

2. The gastric juice owes its destructive power primarily to the hydrochloric acid contained in it, and the damage done to the wall of the stomach varies in proportion to the strength of the acid present.

3. Although the hydrochloric acid is usually only a factor in the initiation of an ulcer, some damage being also inflicted upon the gastric cells by other means, it is occasionally, when of a percentage of 0·39 and upwards and combined with motor insufficiency, the actual cause of the ulcer.

4. The spread of an ulcer is due to persistence of the original cause, but it is more likely to spread when the gastric juice is hyperacid than when of normal acidity.

5. The gastric juice is able to attack the connective-tissue base of an ulcer by virtue of the hydrochloric acid contained in it, and, when hyperacid, to produce various degrees of delay in the healing of the ulcer as a consequence. This effect is added to when retention of food in the stomach or hypersecretion of the gastric juice is present. In this way may be explained the condition of delayed healing of acute ulcer in man.

6. It is probable that in human pathology a complete arrest in healing and a resulting chronic ulcer may be similarly brought about, since the experimental methods available for producing hyperacidity

are not so effective as the condition present in man. It must also be remembered that an ulcer heals with difficulty in proportion to its chronicity, and that the more chronic ulcers may be kept open by the normal gastric juice.

7. An important point in favour of this view is the similarity between the two conditions. The experimental ulcer is delayed in healing by the necrotic condition of its base, brought about by the hydrochloric acid. and it is precisely the fibrotic base covered with necrotic tissue which prevents the healing of human ulcer. The treatment aimed at reducing the acidity of the gastric contents and facilitating the emptying of the stomach is that which is successful in healing a human gastric ulcer.

8. Associated with chronic gastric ulcer in the human subject are commonly the two conditions—(1) hyperacidity and (2) gastritis. The association of the three conditions has in the past given rise to much speculation with regard to the relation which they bear to one another. It is proved by these experiments that continued hyperacidity gives rise to gastritis, and, on the other hand, it is well known clinically that if gastritis affects the acidity of the gastric juice the latter is diminished. It seems reasonable, therefore, to assume that the hyperacidity precedes the gastritis and causes it. Gastritis in ulcer may of course be due to irritants in the food, but the regularity of its occurrence suggests that some constant factor is usually its cause. It is obvious that a gastric ulcer does not give rise to diffuse gastritis, and it is equally obvious from these experiments that follicular ulcers, occurring as a part of gastritis, cannot become chronic by continued hyperacidity. Diffuse gastritis, therefore, is neither the cause nor the effect of the ulcer, but results from the irritant action of the hydrochloric acid, or perhaps in other cases from one of the well-known causes of the affection. We have seen that hyperacidity is concerned as a factor in each of the pathological processes of gastric ulcer, and, on the other hand, it may be stated that, although it has been suggested, proof has never been afforded that an ulcer gives rise to hyperacidity.

9. We thus see the important part which the acid of the gastric juice plays as a factor in the pathological processes of gastric ulcer. It must not, however, be supposed that the importance of this factor is the same in all cases, since there are many different types of ulcer of the stomach brought about by an equal number of different causes. The destiny of an ulcer of the stomach depends upon its cause as well as upon the adverse influences to which it is exposed.

REFERENCES.

1. Katzenstein . . . . *Berl. klin. Wchnschr.*, 1908, Bd. xlv. S. 1749.
2. Wolff . . . . *Samml. klin. Vortr.*, Leipzig, N.F., Innere Medizin, 1909–1910, No. 167–196, S. 315.

3. BOLTON . . . . *Proc. Roy. Soc. London*, 1907, B, vol. lxxix. p. 533: *Ibid.*, 1910, B. vol. lxxxii. p. 233; *Proc. Roy. Soc. Med.* (Path. Sect.), London, 1910, vol. iv. p. 53.

4. MATTHES . . . . *Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1893, Bd. xiii. S. 309.

5. LITTHAUER . . . . *Virchow's Archiv*, 1909, Bd. cxcv. (Folge xix. Bd. v.), Heft 2, S. 317.

6. SUZUKI . . . . *Arch. f. klin. Chir.*, Tübingen, 1912, Bd. xcviii. S. 632.

7. BOLTON . . . . *Journ. Path. and Bacteriol.*, Cambridge, 1910, vol. xiv. p. 418.

DESCRIPTION OF PLATES XVI.-XVIII.

PLATE XVI.

FIG. 1.—Microphotograph of a section of the scar of Experiment 5. The fibrous tissue of the base is covered with a single layer of cubical epithelial cells, which have not yet commenced to form primitive glands. (× 60.)

FIG. 2.—Photograph of the ulcer of Experiment 12, showing the delay in healing on the twenty-first day due to administration of 0·39 per cent. HCl solution. (Slightly reduced.)

FIG. 3.—Photograph of the ulcer of Experiment 21, showing the delay in healing on the twenty-first day due to administration of 0·28 per cent. HCl solution, together with motor insufficiency. (Slightly reduced.)

FIG. 4.—Photograph of the ulcer of Experiment 22, showing the delay in healing on the twenty-first day due to administration of 0·39 per cent. HCl solution, together with motor insufficiency. The slough covering the base is seen to have shrunk from the edges of the ulcer. (Slightly reduced.)

PLATE XVII.

FIG. 5.—Photograph of the stomach of Experiment 23, showing a large ulcer in the centre which was formed by the gastrotoxin and which shows on the sixteenth day very little tendency to heal. The oval ulcer to the left is an acute spreading ulcer which has arisen as a necrotic patch in the mucous membrane owing to the action of the HCl of 0·39 per cent. strength. Motor insufficiency was present. One or two smaller ulcers were also present. (Slightly reduced.)

FIG. 6.—Photograph of the ulcer of Experiment 24, showing the delay in healing on the twenty-first day due to administration of 0·9 per cent. HCl solution, together with motor insufficiency. (Slightly reduced.)

FIG. 7.—Photograph of monkey's stomach, showing gastritis and follicular ulceration due to administration of 0·9 per cent. HCl solution. (Natural size.)

FIG. 8.—Photograph of monkey's stomach, showing circular patches of gastritis due to administration of 0·5 per cent. HCl solution. (Natural size.)

PLATE XVIII.

FIG. 9.—Microphotograph of section of stomach of Experiment 22, showing gastritis due to administration of 0·39 per cent. HCl solution. There is round-celled infiltration of the mucosa, destruction of the gastric glands, and desquamation of the surface epithelium. (× 80.)

FIG. 10.—Microphotograph of section through an enlarged follicle in the mucous membrane of a monkey's stomach, resulting from the administration of 0·5 per cent. HCl solution. A follicular ulcer is forming. The enlarged follicle has burst through the muscularis mucosæ and appeared at the surface of the mucous membrane. (× 75.)

Fig. 11.—Microphotograph of section of cat's stomach, mentioned on p. 154, showing the formation of a necrotic ulcer. The mucous membrane has gradually disappeared almost to the level of the muscularis mucosæ, which is covered with necrotic tissue, embedded in which are the remains of gastric glands. (× 60.)

Fig. 12.—Photograph of monkey's stomach, mentioned on p. 154. The ulcer is seen to occupy most of the anterior surface of the stomach, the base being formed of a large area of the liver covered with slough. (Reduced by about one-half.)

Article No. 3

158

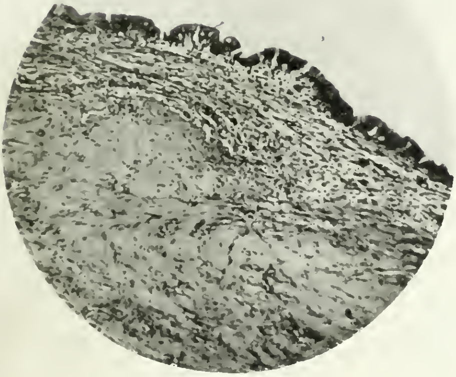


FIG. 1.

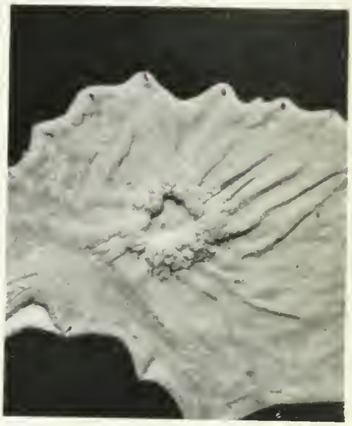


FIG. 2.

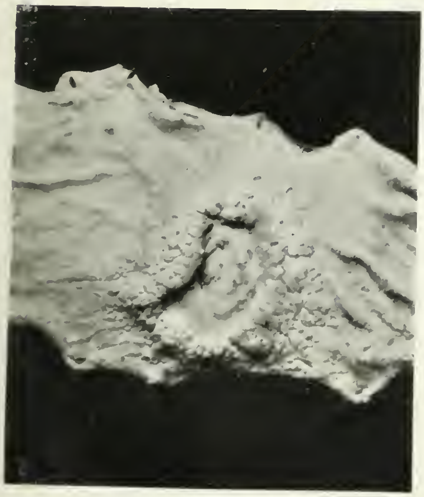


FIG. 3.

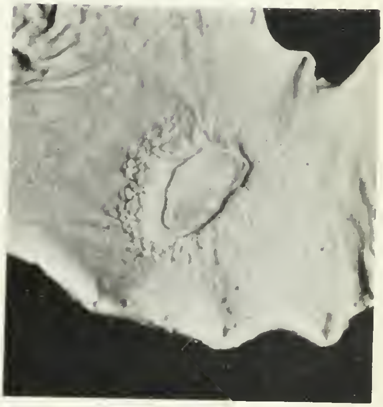


FIG. 4.

1582

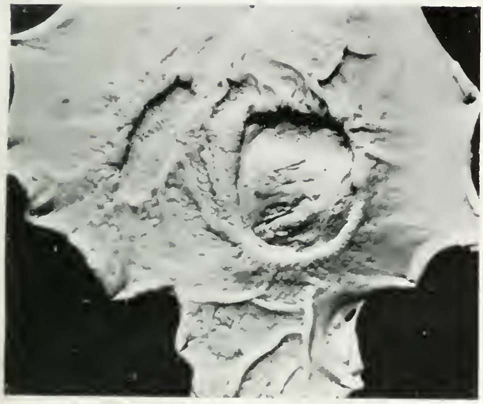


Fig. 5.

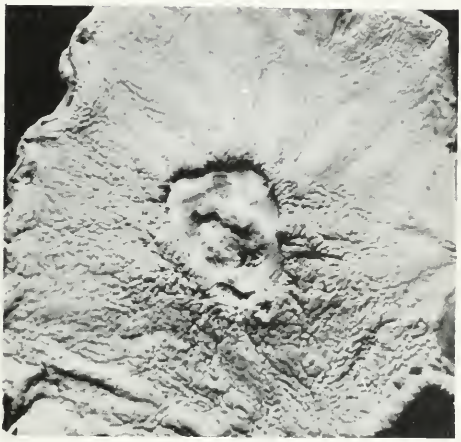


Fig. 6.

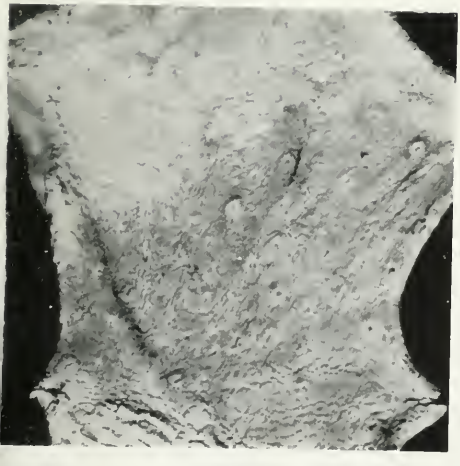


Fig. 7.

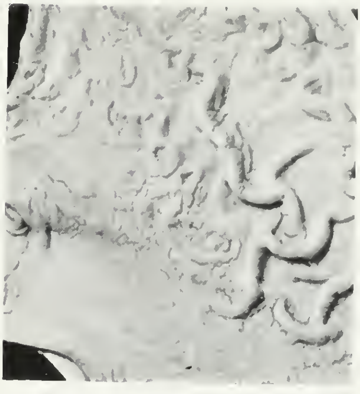


Fig. 8.

1583

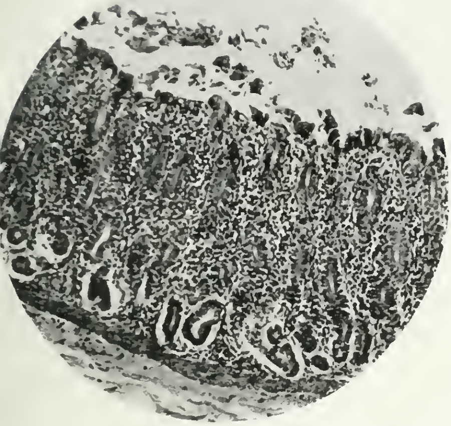


Fig. 9.

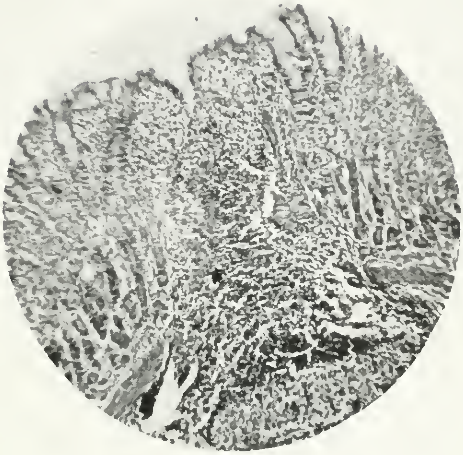


Fig. 10.

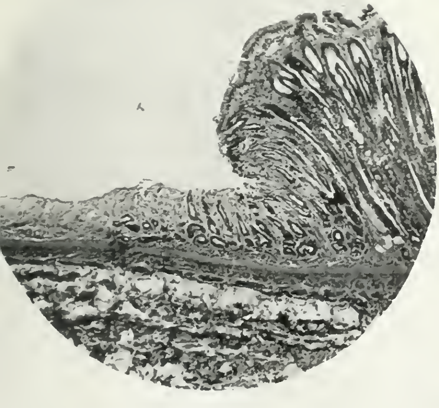


Fig. 11.

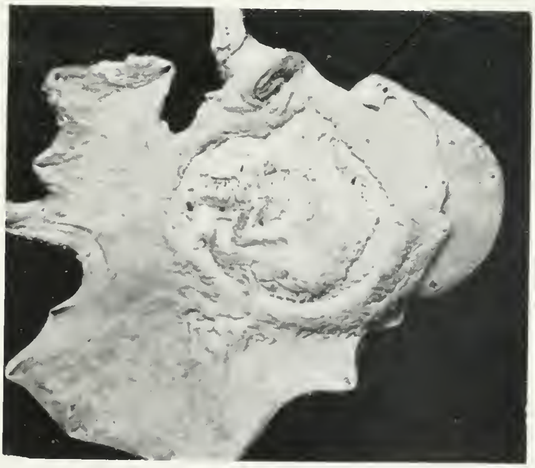


Fig. 12.

38

1c·4

[*Reprinted from the Journal of Physiology,*
Vol. XLIX. Nos. 1 *and* 2, *December* 22, 1914.]

SOME RESULTS OF EXCISION OF THE ADRENAL GLANDS. BY T. R. ELLIOTT, M.D.

(*From the Research Laboratories of University College Hospital Medical School.*)

THE observations, which are briefly narrated in this paper, have been made at intervals during the last twelve years. All the experiments were made on the cat, an animal which invariably dies after excision of its glands. My wish was to analyse the manner of this death, and especially to see whether the end comes with a general failure of the nerve and muscle machinery of the sympathetic nervous system, inasmuch as the medulla of the gland is so closely related with that limited division of the nerves of the body.

The glands were always removed in their entirety by a careful dissection through a lumbar incision without opening the peritoneal cavity. Damage to the gland was avoided, so that it could be weighed on removal and, if required, examined histologically.

Survival time. Excision of a single gland was never attended by the least outward disturbance of the animal's health. Most animals, including man, can live perfectly well with a single adrenal gland. Section of the splanchnic nerves on the side of the remaining gland does not obviously affect the animal's health. It has been abundantly proved that these nerves control the rapid discharge of adrenalin from the medullary cells, and that after their section the adrenalin is retained in a persistently high load[1]. Hence it follows that the sudden outpouring of adrenalin into the blood stream, which occurs with excitation of the splanchnic nervous system, is not essential to life. This fact has already been proved, though less directly, by several workers who found that a dislocated or transplanted adrenal gland sufficed to keep an animal alive.

Complete excision of the second gland leads in the cat practically always to death. Therefore life depends either on a continual slow excretion from the medullary cells or on some activity of the cortical tissue. In a series of 25 cats the glands were removed successively, the second being excised at intervals varying from 3 weeks up to 9 months after the first. Only one cat recovered completely so as to seem capable of living on indefinitely. It was examined and killed nine weeks after the second operation. The entire 25 fell into two main groups. In the first group, death occurred with the usual features early on the second or third day, a time almost as short as that after simultaneous excision of both glands. Nine cats belonged to this group, and in these as a rule the second gland was obviously hypertrophied, the enlargement being almost entirely of the cortex. In the second division of 16 animals, life was prolonged generally for 6 to 10 days, though two survived to the 22nd and the 23rd day, and one as stated above, seemed to have recovered completely when it was killed in the 9th week. In these, the second gland rarely showed any enlargement, but small accessory adrenals of cortical tissue were found to have hypertrophied elsewhere. Hence one could generally predict the time of survival of the cat by observing whether the second gland was or was not enlarged by compensatory hypertrophy.

---

[1] Elliott. This *Journal*, XLIV. p. 374. 1912.

In another series of 21 cats, both glands were excised at one and the same operation, and 19 died in from 18 to 48 hours (generally on the second day). Recovery from the ether was prompt; there was no shock, and for the first few hours the animal would appear perfectly strong and drink milk. Then the fatal weakness developed. Its onset was delayed a little by keeping the animal in a warmed cage so that its rectal temperature did not sink below 100°F. Only two cats survived to the 9th and 10th day respectively. The time was unaffected by previous or simultaneous section of the splanchnic nerves.

Nature of the death. The phenomena were very similar whether the glands had been removed simultaneously or successively. The description given is drawn chiefly from animals in which the glands were removed in two operations, so that the longer survival gave a better opportunity for their examination.

All the cats lost weight, and those which survived longest became extremely thin. This was partly due to a loss of appetite; meat being generally refused, and milk taken only in small quantities and without much relish. Post mortem the gastric juice had its normal acidity, and frequently two or three acute ulcers were found to be present[1], a token of the full digestive power of the juice.

---

[1] This ulceration has been described also by Finzi. *Virchow's Arch.* ccxiv. p. 214. 1913. Ulcers are not seen in human patients with Addison's disease.

The approach of death was always announced by the development of a very characteristic weakness. When placed on the floor, the cat would walk a few steps and then stand switching its tail as though bothered by the difficulty which it found in walking. Soon it would lie down prone with chest and belly on the ground and the limbs rather relaxed. Later, in sheer feebleness it slid over from this position and lay on its side with limp legs and tail fully extended, an attitude never adopted normally. Emotionally it showed no annoyance, and would purr in a languid way until near the final collapse. The eye-balls lost their tension and became soft as the blood-pressure steadily fell. Special care had to be taken by artificial warmth in the cage to check the fall of body temperature, for otherwise the rectal temperature would have dropped from 103° or 104°F. to 85° or 90°F. ere death occurred. Convulsions were very rarely seen.

Yet despite this characteristic prostration and weakness the animal could be roused by alarm to fully co-ordinated and fairly strong muscular movements. There was no paralysis of the skeletal muscles or their nerves. This was very evident when the moribund cat was etherised for experimental examination. The anæsthetic was given under a large glass bell jar, and here so soon as the cat smelt the ether it moved almost as freely and quickly as a normal animal would have done. There was also the usual free salivation. An equally convincing proof of the full control of the skeletal muscles by the central nervous system was given by the appearance of decerebrate rigidity. The blood-pressure of the dying animal was extremely low and it rose very little in the struggles attendant on etherisation. But often this slight muscular effort would cause the heart to fail still further and then, as the pressure sank to 10 or 15 millimetres and could no longer supply blood to the cerebral hemispheres, deep, gasping respirations would ensue and the fore limbs of the cat set stiffly in an extensor rigidity, while sometimes the hind limbs commenced an automatic walking rhythm. The intravenous injection of a small amount of adrenalin restored the blood-pressure, and with the fuller circulation the extensor rigidity disappeared rapidly. The sensitiveness with which this tonic control reacted to changes in the blood-pressure proved that all the nervous connections of the trunk musculature were in a practically normal state. To the relatively coarse test by faradisation of the sciatic nerve the leg muscles responded with the usual contraction.

The mechanism of the heart beat appeared to remain unaltered during the gradual failure of the circulation until death. In one cat, which survived double extirpation for 14 days, electrocardiograms were kindly taken for me by Dr Thomas Lewis on successive days until the animal fell into its final collapse. These showed no variations from the normal curves.

The visceral nerves. No paralysis was observed in the cardiac fibres of the vagus. A faradic stimulus of minimal strength would slow the heart in the usual way. Reflex control was exerted also as in the normal cat, so that the heart beat faster when the vagi were cut, and especially so after the circulation had been stimulated by a small dose of adrenalin (see Fig. 1). The light reflex of the pupil through the third nerve was normal.

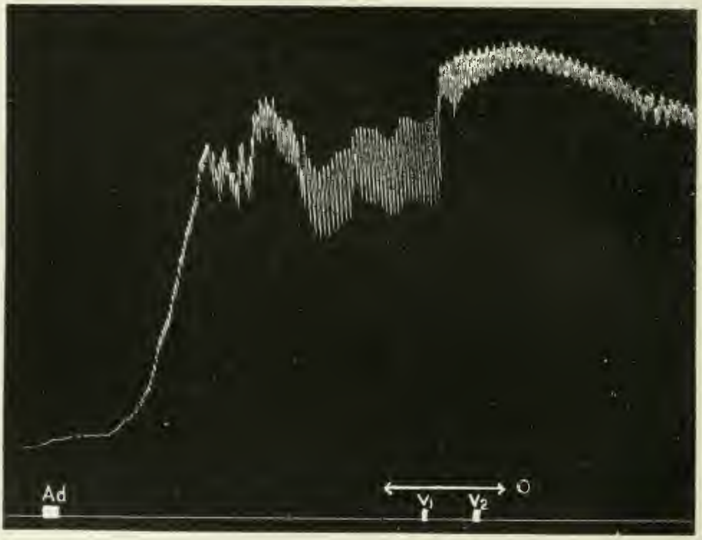


Fig. 1. Both adrenals excised 46 hours. Rectal T. 96°. Ether. Bl. pr. 16, and heart beat imperceptible. Adrenalin, 0·4 mgm., injected into external jugular to restore life. Bl. pr. rose rapidly and heart beat became very slow at 120 mm. Each vagus was cut in turn at the points marked. The heart escaped from vagal slowing, and the pressure rose to 170. Later the cat showed well marked decerebrate rigidity in the fore legs and walking movements in the hind legs. The splanchnics were paralysed. Unless otherwise indicated in the tracing, the zero of bl. pr. is in all these figures given by the signal time. All the figures are reduced to $\frac{2}{3}$ original size.

The pelvic visceral nerve to the bladder and rectum was perfectly efficient. A moribund cat, despite its low blood-pressure, would sometimes void urine very powerfully in the early stages of anæsthetisation. Stimulation of the pelvic nerve caused the usual contraction of the bladder wall. And it was always found in cats which were allowed to die quietly without experimental examination, that the bladder was contracted and empty, in contrast with the dilatation and engorgement of the splanchnic blood vessels.

On the other hand a very real paralysis could be demonstrated in some of the sympathetic or thoracico-lumbar nerves. This requires detailed discussion. The *afferent* nerves could apparently convey impulses as usual from the viscera. Certainly there was no loss of sensation in the cutaneous afferents. To prove that the visceral afferents were also functionally active was less easy, because blood-pressure reflexes were annulled by the failure of the efferent path. In a normal cat, when fully anæsthetised with ether, the stimulus caused by snipping with scissors the splanchnic nerve within the thorax is always so strong as to cause a reflex jump of the animal's body. Exactly the same reaction was observed when the splanchnic nerve was cut in these moribund cats under ether. Therefore the visceral afferents could convey impulses of the usual reflex power.

On the *efferent* side the paralysis was restricted to the vaso-motors. The other sympathetic nerves did not show any notable loss of power: thus the cervical sympathetic would produce all its usual effects on the eye when stimulated by a minimal faradising current. The pilo-motors were also fairly efficient; this corresponds with what I have observed clinically, that in man in the last collapse of Addison's disease the pilo-motors can still be easily excited to a prompt "goose skin" reflex by exposure or by rubbing the skin.

In order to test the vaso-motor nerves the splanchnics were cut and stimulated both together within the thorax. This method avoids all interference with the abdominal viscera. When the cat has yielded to the final weakness its blood-pressure is extremely low, 20 to 40 mm. under ether instead of the usual 120 to 160 mm. So poor then is the circulation that there is no bleeding from the small vessels in the pre-tracheal muscles during tracheotomy. Under these conditions the splanchnic nerves yield even with strong stimulation a slow rise of only 10 or 20 mm. Such failure might be due to a block in the ganglion cells, or to a loss of reaction in the peripheral tissues, either at the junction of nerve and muscle or even in the contracting muscle itself.

Nicotine, or cytisine, causes in the normal cat, especially after a preliminary injection of atropine to prevent inhibition of the heart, an abrupt rise of pressure to over 200 mm. This is the result of a simultaneous stimulation of all the vaso-constrictor ganglion cells. In the cat that is dying after loss of its glands the rise is only 10 or 20 mm. high. If atropine has not been injected there may be actually only a fall from the vagal slowing of the heart (see Fig. 2). From this it follows that the vasoconstrictors and the cardiac accelerators are rendered impotent, either at the ganglion cells or somewhere nearer to the periphery.

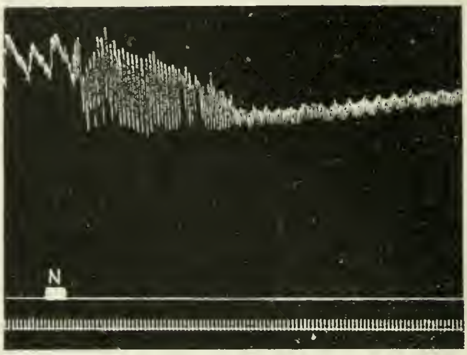


Fig. 2. From same cat as Fig. 1. The experiment had been in progress 70 minutes, and 2·2 mgm. adrenalin altogether had been injected so that the bl. pr. was improved. Nicotine, 5 mgms., injected; bl. pr. fell from 80 to 60 mm. with slowing of heart: strong muscular spasms of body.

Pituitary extract and barium chloride as is known normally cause a prolonged rise of blood-pressure, the first developing slowly while the second commences as abruptly as that by nicotine; and it has been proved that in each case the stimulus acts directly on the plain muscle of the blood vessel wall. On the circulation of these moribund cats the drugs are practically without effect (see Figs. 3 and 5) and direct inspection after the injection of nicotine or barium chloride reveals that the blood vessels do not contract either in the intestinal wall or in the spleen. From this it

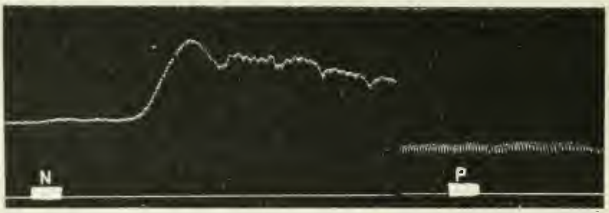


Fig. 3. Failure of barium chloride and of pituitary while nicotine and stimulation of the splanchnics were still moderately effective. Both glands excised, left 56 and right 6 days. Cat still fairly strong: rectal temperature 102°. Bl. pr. 80, falling after section of splanchnics in thorax. Stim. splanchnics, rise from 48 to 60. Atropine ·4 mgm. At *N* inject 4 mgm. nicotine, rise of bl. pr. 40 to 70, and strong contraction of bladder, colon, and ears. At *P* inject ·5 c.c. pituitary extract, bl. pr. unaffected. Later adrenalin raised bl. pr. from 20 to 160, but barium chloride was as ineffective as pituitary extract.

would appear that the paralysis of the splanchnics is not due to any fault in the nerves themselves, but arises simply from a loss of contractile power in the muscles of the blood vessels and perhaps of the heart.

Yet even in this general failure adrenalin can still act. A quantity little larger than that needed in a normal cat will raise the blood-pressure to 150 or even higher, while the heart beats with full vigour again. The muscle is therefore still capable of contraction. Repeated injections of adrenalin do not uphold the level of the blood-pressure indefinitely, nor do they bring back the power to react to nicotine or splanchnic stimulation. Some experiments may be quoted in detail to illustrate these general statements.

The vaso-motor paralysis was found only in the very weak and moribund animals. If the cats were examined soon after excision of the adrenals and before they had fallen to this degree of collapse, their blood vessel reactions were found to be almost normal. Of such a condition the first three experiments furnish examples.

Exp. 1. Large entire ♂ cat, 3200 gm. Right gland excised = ·20 gm. 7½ months later, weight 3500 gm.; left gland excised, = ·23 gm. Examined under ether 9 weeks later, when cat was in fair health, though thinner, 3050 gm. There was no Addisonian pigmentation.

Bl. pr. 170 mm., falling to 70 after section of splanchnics in thorax.

Stim. both splanchnics in thorax; bl. pr. 60 to 110, and upheld type of curve without adrenalin drop[1].

Adrenalin, ·025 mgm., injected; 76 to 160.

Pituitary extract, ·5 c.c. of 20 %, injected; 100 to 160 to 130.

Killed. Three accessory cortical glands found along the spermatic veins below the kidney, only one of which contained a trace of chromaffine cells: total weight of the three = ·065 gm.

Abdominal paraganglia of normal size. Thyroid and thymus normal.

Pituitary small = ·038 gm.

Exp. 2. Both adrenals excised at once. 18 hours later, fairly strong and can sit on haunches.

11.27. Etherised. Bl. pr. 40. Lively reflexes on pinching tail, but bl. pr. unaffected in consonance with these movements.

11.40. Both splanchnics cut in thorax: no further fall of bl. pr.

11.44. Stim. both splanchnics (Kronecker scale 2000 and 3·5 volts), 40 to 100 (Fig. 4).

11.52. Inject cytisine 5 mgm., bl. pr. 44 to 130.

11.56. Stim. splanchnics, no rise owing to block by cytisine.

11.58. Inject adrenalin ·025 mgm., 50 to 200.

Exp. 3. Pregnant female. Both glands excised. A few hours later she aborted three kittens: 22 hours after operation quite strong. Ether. Bl. pr. 130.

Both splanchnics cut in thorax, bl. pr. fell to 70.

---

[1] Cf. Elliott. This *Journal*, XLIV. p. 402. 1912.

Stim. both splanchnics; 60 to 110.
Inject 5 mgm. cytisine; 100 to 200 to 50.
Inject ·02 mgm. adrenalin; 34 to 240.

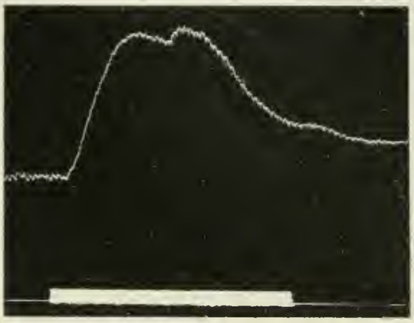


Fig. 4.

The next examples illustrate the more complete paralysis.

Exp. 4. Small, entire ♂ cat, weight 2230 gm. Right gland excised = ·16 gm.

105 days later 3035 gm., the cat having grown to adult size: left gland excised = ·25 gm.

5 days later, moribund, weight 2850 gm., rectal T. 100·2°F. Knee jerks brisk. Very light etherisation.

Stim. cervical sympathetic, full eye reactions.

Stim. vagus, heart slowed, bl. pr. 50.

Atropine 10 mgm. Nicotine 8 mgm. and again 8 mgm. (at *N*, *N′*, Fig. 5), bl. pr. 36: slight slowing of heart and strong twitching of body muscles.

Adrenalin ·025 mgm., bl. pr. 30 to 130 (at *Ad*, Fig. 5).

Pituitary extract, ·5 c.c. of 20%, bl. pr. 40 to 50.

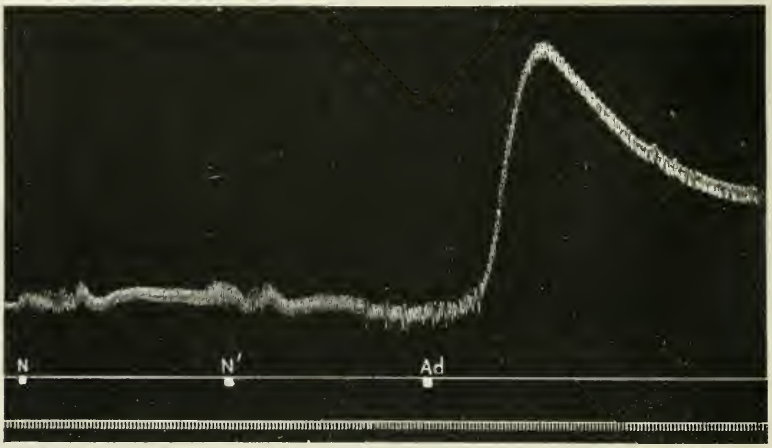


Fig. 5.

Adrenalin ·025 mgm. bl. pr. 40 to 210.

Killed. No accessory adrenals found. Testicles contained a normal load of interstitial fat.

Exp. 5. Both adrenals excised. 14 hours later, morphia 20 mgm.; this made cat angry and very excited all the day. 22 hours after operation, rectal T. 103·5°F. Ether.

Stim. cervical sympathetic, full effect with minimal stimulus on Kronecker inductorium. Bl. pr. 48.

Cut splanchnics, bl. pr. 44.

Stim. both splanchnics, at *S.*, Fig. 6, bl. pr. 28 to 36.

Inject 5 mgm. cytisine at *C* Fig. 6, heart slowed, bl. pr. 20 to 28.

Inject ·02 mgm. adrenalin at *Ad*, Fig. 7, bl. pr. 20 to 180. (Fig. 7.)

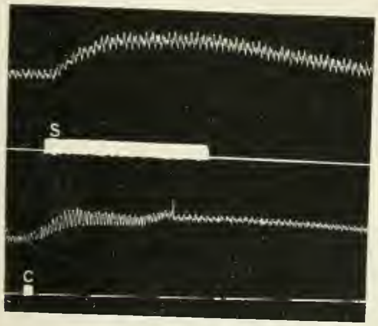


Fig. 6.

Exp. 6. Young cat. Both adrenals excised. Right splanchnic nerve cut. Ten days later, very weak and unsteady on hind legs. Rectal T. 99·5°F. Ether. Bl. pr. 56.

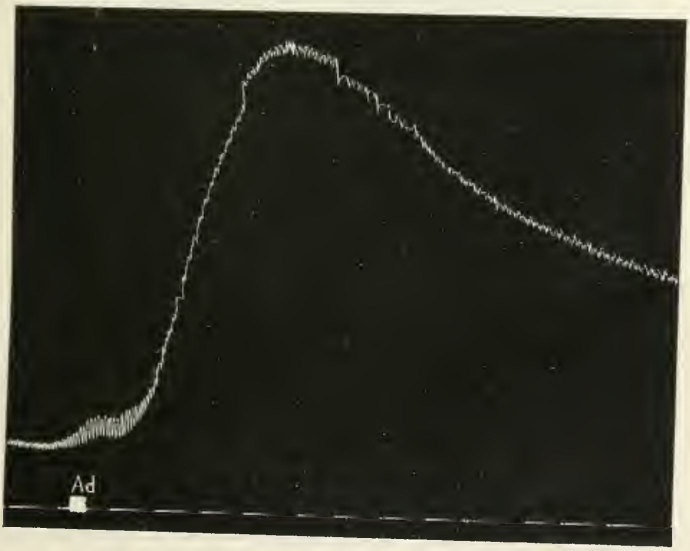


Fig. 7

Stim. right vagus: full inhibition.

Cut left splanchnic in thorax: sensory twitch reflex of body.

Stim. left splanchnic: bl. pr. 50 to 64, but full pilomotor effect on dorsal hairs.

Clamp sciatic nerve: bladder forcibly emptied as a reflex, but no rise of bl. pr.

Inject nicotine, 10 mgm. bl. pr. 30: heart slowed and animal nearly killed.

Adrenal, ·04 mgm., bl. pr. 20 to 180.

Not examined for accessory glands.

Exp. 7. Both adrenals excised. Very weak 24 hours later. Rectal T. had fallen to 95° F. Cat micturated feebly when tied for etherisation. Extensor rigidity then developed and circulation practically ceased.

Adrenalin .02 mgm. quickly injected: cat recovered and respiration commenced again. Again .02 mgm., bl. pr. 44 to 130.

Atropine 1 mgm. bl. pr. 40.

Nicotine 5 mgm., bl. pr. 40, no rise.

Pituitary, 1 c.c., bl. pr. 38 to 48.

Barium chloride 1 c.c. of 2 %, bl. pr. 48 to 60.

Adrenalin .02 mgm., bl. pr. 40 to 130. Cat moves tail and claws in fullest co-ordination.

Nicotine 5 mgm., bl. pr. 40 to 48.

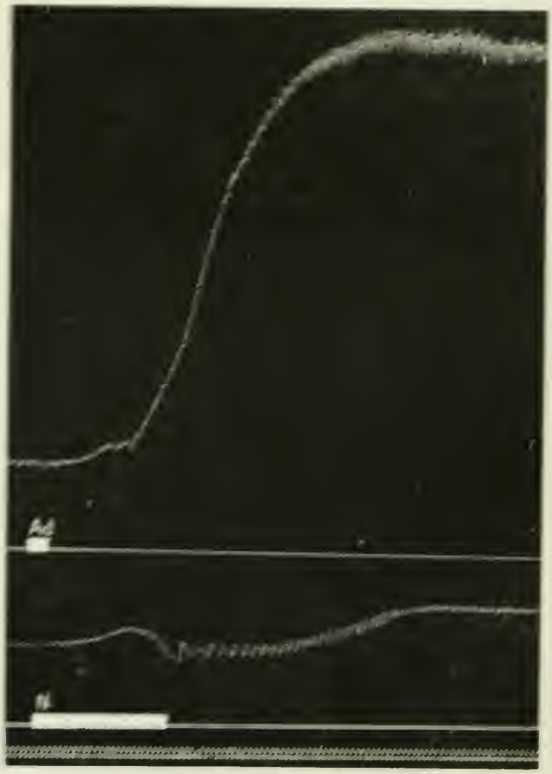


Fig. 3.

Exp. 8. Both adrenals excised, and right splanchnic cut. 30 hours later, rather weak. Rectal T. 98° F.

2.15 Ether. Bl. pr. 28. Artificial respiration needed.

2.30 Cut left splanchnic below diaphragm: bl. pr. 26.

Stim. left splanchnic, Kronecker scale 2000 with 3·5 volts, bl. pr. 26 to 34.

Cut vagi, heart beat accelerated.

Stim. right pelvic, full ipsilateral contraction of bladder.

Stim. cerv. symp., fair reaction.

3.10. Nicotine, 10 mgm. at *N*, Fig. 8, bl. pr. 25 to 20, as heart slowed, and then rise to 30.

3.15. Adrenalin, ·03 mgm. at *Ad*, Fig. 8, bl. pr. 22 to 186.

These last five examples illustrate the paralysis of the blood vessels. Many similar experiments were made and they all gave uniform results, provided that the animal was examined in its last stage of collapse. No reflex or direct stimulation, except that of adrenalin, could elicit a rise of blood-pressure. Cybulski had observed in his original paper that, after removal of the adrenal glands, asphyxia failed to cause its usual rise of blood-pressure by central nervous stimulation and, though this was denied by Schäfer and Oliver, I found his statement to be quite true. It is a further illustration of the final paralysis that is analysed in this paper.

Certain control experiments remain to be quoted. It is almost unnecessary to exclude the suggestion that in the dissection for the removal of the adrenal glands, the splanchnic nerves might themselves suffer direct damage. The nerves were avoided, but even had they been injured the damage could not have accounted for the peripheral loss of reaction to barium or pituitary. As direct experiment showed, the operative procedures, apart from excision of the adrenal glands, caused no appreciable change in the effect of barium chloride, pituitary or other drugs.

Nervous control of vaso-motor tone. The analysis by drugs proves that the loss of vascular tone described above is caused by a peripheral failure and not by a primary paralysis of the vaso-motor nerves. The nerves appear to be paralysed because the muscle cannot respond to their excitatory impulses. When the mass of the vaso-motor nerves are removed, and the blood vessels are left only with the secretion of the adrenals to maintain their automatic tone, an entirely different picture is seen. Section of the splanchnics on both sides causes no apparent interference with a cat's health. The blood-pressure after such an operation was found 20 days later under ether to be 150, and it reacted well to nicotine and to adrenalin.

Exp. 9. Laminectomy, and spinal cord destroyed from the first thoracic segment down to the fourth lumbar. 29 hours later—cat strong, but syncope was at once induced when the animal was raised to a vertical position. This fainting does not occur after excision of the adrenals until a cat is in its final collapse.

Rectal T. 30°. Pre-tracheal muscles full of blood. Bl. pr. 100.

Stim. left spl., bl. pr. 84 to 166.

Nicotine 10 mgm., bl. pr. 92–22–206.
Adrenalin ·018 mgm., bl. pr. 70 to 172.

Exp. 10. Both stellate ganglia removed by dissection through posterior thoracic wall. 23 days later splanchnics cut and all the mass of the semilunar ganglia removed. Recovered perfectly: no syncope, when placed in the vertical position.

9 days later etherised. Heart beating strongly, bl. pr. 140.

Vagi cut, rise to 190. Stim. splanchnics, no effect.

Atropine 5 mgm., bl. pr. 140.

Nicotine 4 mgm., bl. pr. 130 to 190, the rise being very slow, perhaps in consequence of the removal of so many vaso-constrictor ganglia.

Pituitary extract, ·5 c.c., 110 to 90 to 170.

Adrenalin, ·025 mgm., 120 to 220.

Barium chloride, 1 c.c. of 2·4 %, 150 to 190.

Killed. (Ileo-colic sphincter was found to have been fully competent.)

Exp. 11. At one operation there were excised both stellate and the semilunar and inferior mesenteric ganglia. Weight 3000 gm. In the next seven days the animal drank only a little milk and lost 1000 gm. in weight, though it walked about with fair strength. Etherised. Rectal T. 102·5° F. Bl. pr. 108.

All the muscles were full of blood, unlike the dry, anæmic state of the tissues in the glandless cat. Intestines not congested, but they showed increased peristalsis, and blanched and contracted with unusual readiness in response to a local touch.

Bl. pr. fell to 60 as soon as the viscera were exposed and remained henceforward at this low level.

Adrenalin, ·01 mgm., 54 to 114. Killed. Adrenals weighed ·26 and ·30 gm., being loaded with chromaffine substance.

It has been shown by Schiff, Ewald, Goltz and others that the tone of the blood vessels is not derived entirely from their connection with the peripheral ganglia or with the central nervous system. In the experiment given above it is seen that after severance from the major part of the visceral ganglia, and after destruction of the central nervous system control of the entire vaso-motor system, the blood vessels gave a full reaction to barium, pituitary, or adrenalin, though the effect of nicotine was naturally modified by the loss of the peripheral ganglion cells, which lessened the extent and the abruptness of the rise.

Shock and lowered temperature. Insomuch as the atonic paralysis cannot be demonstrated except in the final collapse of the animal, it might be urged, as indeed it has been by Hoskins and Wheelon,[1] that this is only such a terminal phenomenon as may appear in any condition of shock and collapse, and that it is not a peculiar result of the absence of the adrenal glands. To this an obvious reply would be that even were the phenomena of fatal shock and those consequent to loss of the adrenals to be identical, yet that would only suggest that the cause in each is the same, namely loss of adrenal secretion. It has been proved that severe shock does exhaust the adrenal glands to a profound degree. But the phenomena are not altogether the same. In moderate shock there is very little paralysis.

---

[1] *Amer. Journ. Physiol.* xxxiv. p. 172. 1914.

With greater shock there is fall of temperature and greater vasomotor paralysis. Now fall of temperature in itself produces some degree of paralysis, and especially in the circulatory muscles. This can be studied by placing an etherised animal in iced water until the rectal temperature falls to 85° F. (29° C.)[1]. All blood-pressure rises are then lessened and delayed, equally whether it be to adrenalin or to nicotine. But there is not so much paralysis as after excision of the glands.

Exp. 12. Large cat. Etherised.
3.40. Cut splanchnics in thorax.
3.50. Atropine, 10 mgm. Rectal T. 102° F.
3.54. Stim. both splanchnics, bl. pr. 130–170–150–200.
4.5. Cooled in iced water.
4.35. Rectal T. 85°. Adrenalin, ·025 mgm., bl. pr. 90–184.
5.15. Rectal T. 84°. Stim. both splanchnics, 84–140–110–120.
5.20. Nicotine, 6 mgm., 130–175.
5.27. Barium chloride, 1 c.c. 2·4 $^0/_0$, bl. pr. 140–190.

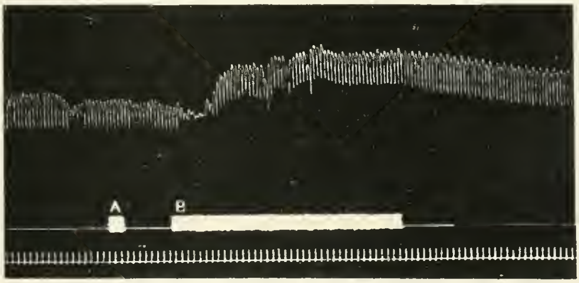


Fig. 9.

In this last example the animal was cooled for only one hour. When the temperature is kept at an abnormally low level for a longer time the blood-pressure does not fall any lower, but it reacts slowly to stimuli, while the heart beat also becomes unusually slow. This factor of low temperature certainly entered into the next example of profounder shock.

Exp. 13. Left stellate ganglion excised under chloroform. Twenty hours later the animal had not recovered and now lay utterly collapsed, cold, and apparently lifeless; respiration very slow. Rectal T. 94° F.

---

[1] Cf. Elliott, This *Journal*, xxxii. p. 452. 1905.

Ether. Bl. pr. 60, with slow, big heart beat, uninfluenced by section of vagi. Cut both splanchnics at *A*. Stim. both at *B* (Fig. 9), 56 to 70.

Nicotine 5 mgm., bl. pr. 46, heart slowed still more.

Adrenalin ·02 mgm., 44 to 108.

It must be granted, therefore, that shock and cold can produce changes in the blood vessels which are not unlike those consequent to removal of the glands. But shock does not explain the phenomena seen in the latter case, for the cats recovered perfectly after the operation and showed no untoward symptoms until the fatal weakness developed. Shock, to be such, must be in evidence at once after the operation and it must develop its paralysing collapse progressively to the end.

Nor can cold be accepted in explanation, for the use of artificial heat prevented the temperature in the glandless cats from falling more than 5° F.

Cortex of the gland. As stated above, compensatory hypertrophy is an overgrowth of the cortex rather than of the medulla. The accessory glands, which may enlarge in this reactive process, are composed almost entirely of cortical cells. Exp. 1 suggests that they may be enough to provide what is needed for the maintenance of life. But compensatory hypertrophy is variable and it often fails to take place after removal of one gland.

In addition to the share that they probably take with the medulla in providing the means needed to maintain the cardio-vascular tone, the cortical cells do undoubtedly preserve a remnant of some old ancestral power which influences the growth of the animal in respect of its sexual attributes. Of this physiology has as yet discovered no evidence. But the proof is sufficiently convincing on the side of human pathology, where facts lie to demonstrate what might, indeed, be surmised from the arguments of morphology. Large tumours of the adrenal cortex cause the human body to acquire the characters of adolescence years before the proper epoch of puberty. In addition to this premature setting of the body, there is in such cases a strong tendency for the features of maleness to be exaggerated, and even to submerge those of femaleness with which the body was endowed before the cortical tumour commenced to grow. By the kindness of Dr Gordon Holmes, I was able to observe a striking example of this change. A young woman, who had developed in a perfectly normal way until the age of twenty, found that her catamenia then ceased. Hair soon commenced to grow on her face; and four years later she was bearded, and the muscles of the lower half of her body showed a masculine contour. The menses had never returned. Emotionally she was still female. A few months later a tumour could be felt on the right side of the abdomen, and on removal this was found to have arisen from the cortex of the adrenal. Soon after the operation the menstrual flow recommenced, but sufficient time has not yet elapsed to show whether her body will regain its femaleness, and so complete the argument.

The adrenal cortex then contains some element that governs the maleness of the body. Embryologically its cells are derived from a tissue lying close to the kidney, from which the sex gland also originates. As the latter descends in the abdomen it carries with it along the spermatic veins small islets of this tissue, which later may develop to form the accessory adrenals that have been described in these experiments. The interstitial cells of the testis, which are laden in many animals with fat resembling that of the adrenal cortex, are therefore related morphologically to the latter.

I examined the testicles and the ovaries of cats which had survived single and double extirpation of the adrenals for a long time. They showed no abnormality. Conversely, I found that ovariotomy (three cases for one year) and castration (nine cases, interval unknown) produced no correlated changes in the weight of the adrenal glands. The adrenals of cats vary considerably in size from individual to individual. A series of 85 cats was taken, and the weight of the glands tabulated in the scale of body weight. The glands of the cats, which had been castrated or ovariotomised, fell within the normal limits of divergence for this series. Thus the experiments failed to give evidence of interchange of function between the interstitial cells of the testis and the cortical cells of the adrenal gland.

SUMMARY.

Removal of one adrenal gland in a cat does not obviously affect the animal's health, nor does it when in addition the splanchnic nerves on the opposite side are cut. It causes usually some hypertrophy of the remaining adrenal, and of accessory adrenals. The hypertrophy is almost entirely of cortical tissue. Subsequent removal of the remaining gland practically always causes death, but death does not usually follow as quickly as when the two glands are removed at one operation; this is probably a consequence of the hypertrophy mentioned above. In

the moribund cat the blood-pressure is low, and there is nearly complete paralysis of the vaso-motor and cardio-accelerator nerves (all other nerves reacting in an approximately normal manner). Thus stimulation of the splanchnic nerves, and injection of nicotine, cause very slight rise of blood-pressure. The paralysis is due to a change in the unstriated muscle, since pituitary extract and barium chloride have little or no effect. On the other hand, a rise of blood-pressure and a quickening of the heart beat are still caused by adrenalin. The paralysis of vaso-motor nerves is not due to shock. A sufficient decrease of temperature causes a somewhat similar paralysis, but the decrease of temperature in the glandless animal is insufficient to account for the paralysis found. The paralysis is then a change due to the absence of adrenal secretion.

Some evidence is given that the cortical adrenal cells influence sex characters.

Towards the expenses of this work a grant was received from the Graham Research Fund of the London University.

No. 5

pp. 54–66

[*Reprinted from the Journal of Physiology,*
Vol. XLIX. Nos. 1 *and* 2, *December* 22, 1914.]

THE RELATION BETWEEN SUCCESSIVE RESPONSES OF THE VENTRICLE TO ELECTRIC STIMULI AND VENTRICULAR FIBRILLATION. By A. G. LEVY.

(*From the Cardiographic Department, University College Hospital Medical School*[1].)

I HAVE recently had under consideration the relation of complex ventricular tachycardias (such as are the outcome of the action of adrenalin upon the mammalian heart under chloroform) to ventricular fibrillation, and the following investigation was originally undertaken in order to ascertain if similar processes were involved as a result of faradization of the ventricle. The processes involved, as it turns out, are in some respects dissimilar, but the results obtained possess considerable intrinsic interest, especially when considered in the light of recent observations made by G. R. Mines[2] upon the conditions attending the effect of successive induction shocks upon the frog's heart. If the ventricle be excited by a rapid succession of electric stimuli it does not respond to every stimulus, for instance every second or third stimulus alone may elicit a response, but if the stimuli be applied at first at a slow rate, and the rate very gradually increased, then the ventricles may be made to respond to every successive stimulus. With the increase in the rate of stimulation certain changes in the electrical responses are noted, which make the increased rate of contraction possible, viz. (1) the wave of excitation lasts a shorter time at any one point in the muscle, (2) the rate of propagation of the wave of excitation becomes slower. "The wave of excitation becomes shorter and slower. Similarly the refractory phase (towards strong induction shocks) is shortened." From these observations Mines argues that a wave of excitation may become so slow and so short that the excited state will have passed off at the point of stimulation, and have left it again highly excitable, before the wave has passed around the ventricle and back to its point of origin; under such circumstances the wave may spread a second time over the same

---

[1] Towards the expenses of this work a grant was received from the Graham Research Fund, University of London.

[2] This *Journal*, XLVI. 349. 1913.

tract and may continue to circulate thus independently of any stimulus. He suggests that such a circulating rhythm may constitute the condition known as ventricular fibrillation, and that in this way a connection is established between a rapid succession of ventricular responses and ventricular fibrillation.

Methods. The animals employed were cats. The anæsthetic was either chloroform or urethane and ether. In a few cases anæsthetisation by cerebral pithing was performed. In some of the experiments an initial intravenous dose of atropine sulphate (3 mgms.) was injected. The volatile anæsthetics were administered through regulating inhalers.

The thorax of the animal was opened and the pericardium incised to allow of the application of a pair of fine fish-hook electrodes to one of the ventricles; the electrodes were connected by a thin cable to the induction coil, and the chest walls were then brought into apposition. A signal to record the individual shocks was introduced into the circuit of the primary coil. The strength of the shocks was generally adjusted so as to be fully efficient in evoking ventricular responses but not unnecessarily strong. The records were for the most part electrocardiographic, the lead adopted being the right fore-leg, left hind-leg. The time was marked in intervals of $\frac{1}{5}''$ and $\frac{1}{25}''$ by means of a Bull rotary time marker. A Lucas' comparator was used to measure small intervals in the graphic records.

The rate of the induction shocks was regulated by means of a Gaiffe interrupter, to which a fine screw adjustment was adapted for the purpose of producing gradual and progressive variations; a long index hand with a graduated arc served to afford a rough guide to the rates employed. This form of interrupter is not entirely satisfactory, for the rate of vibrations does not always remain constant, the fluctuations being sometimes considerable; for this reason my investigations have been in some respects limited. At the onset of the faradic current the rate of shocks is always inconstant, but after a little interval the rate may become fairly or completely constant; the vibrator was therefore always set going for a time before actually stimulating the heart. The shocks were thrown into the ventricle by a double key which made both the circuit to the electrodes and another circuit to a signal, worked by a separate battery, at the same moment. This signal was especially useful for indicating the exact moment of cutting out the current.

In another series of experiments in which two pairs of electrodes were employed, through which shocks were delivered in alternate succession, a different form of interrupter was employed. A disc was rotated at an adjustable rate by means of a friction gear driven by an electric motor. A projecting stud on the periphery of the disc broke in succession two contacts at equal intervals of time. These two contacts were connected with separate induction coils, and the two coils were adjusted to give as nearly as possible the same strength of stimulation. Owing to the want of fine adjustments for spacing and regulating the rate of shocks, this method was not always completely satisfactory.

(*a*) *Blood-pressure effects.* A succession of responses to induction shocks applied to either ventricle is accompanied by a reduction of the blood-pressure; the more rapid the responses the lower the pressure becomes, until eventually it falls to a virtual zero point, the ventricular responses being then entirely ineffectual as a driving force. The succession of muscular responses at or near this extreme stage presents to the eye the spectacle of a rapid quivering of the muscular walls, similar to that of the fibrillating ventricles, but it is differentiated from the latter condition by the immediate resumption of a normal rhythm and a consequent rise of blood-pressure on cessation of the stimuli.

Five experiments were performed in this branch of the investigation, a Hürthle manometer being employed in conjunction with a damped mercury manometer, both connected with the same carotid artery, the curves being registered on a kymograph. The results were substantially the same in all the animals—they did not appear to be materially affected by the form of the anæsthetic or by the injection of atropine. Exp. 1 will serve as an example.

Exp. 1. Cerebral pithing. Electrodes applied to left ventricle. Normal blood-pressure = 90 mm. The results may be expressed in tabular form thus:

	Rate of excitations	Rate of responses	Blood-pressure during excitations
1.	340	340	38 mm.
2.	420	210	54 ,,
3.	570	285	46 ,,
4.	720	360	39 ,,
5.	360	360	36 ,,
6.	510	510	27 ,,
7.	720	360	38 ,,
8.	1320	Nil, fibrillation	14 ,, (virtual zero)

Such results are in conformity with Mines' premises. As the ventricle responds in increasing rate to a sequence of electric stimuli the contraction wave becomes shorter and slower in rate of propagation, so that the ventricle becomes less and less uniformly contracted, and eventually only a portion of the ventricular wall is in a state of maximum contraction at any one moment. The ventricular contraction becomes therefore less and less effective with increasing rates of responses, and the blood-pressure gradually falls. During fibrillation the blood-pressure is invariably reduced to zero.

(*b*) *Electrical effects.* A number of records were obtained from a single animal which illustrate very well certain effects of a relatively low rate of faradic shocks applied to the ventricle (Exp. 2, Fig. 1).

Exp. 2. Chloroform, 1 $^0/_0$ throughout. Electrodes applied to the left ventricle at its base, near the septum; the deviations of the electric response to the stimuli are in a downward direction in the electrocardiograms. In the figures *A* = cut out signal. *B* = electrocardiographic curve. *C* = Hürthle curve. *D* = shock signals. Time marked in $\frac{1}{5}''$. Reduction to $\frac{4}{5}$.

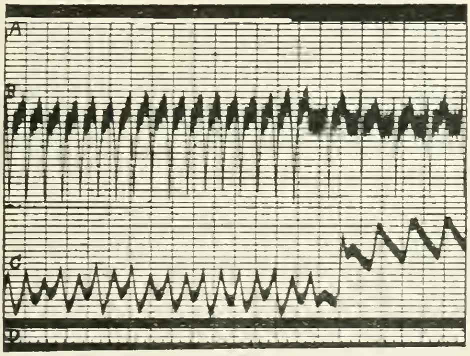


Fig. 1 *a*.

Fig. 1 (*a*). Rate of excitation, 710 per minute; response of ventricle to alternate shocks at a rate of 355 per minute. The blood-pressure is notably depressed. On cessation of the excitation the ventricles immediately become inactive and after a short pause assume a spontaneous beat; the blood-pressure recovers at the same moment.

Fig. 1 (*b*) shows a higher rate of ventricular response. Excitation rate = 930 per minute, response rate = 465 per min. In this case likewise the ventricle becomes inactive on cessation of the excitations and after a pause resumes a perfectly normal beat in response to auricular contractions. It will be noted that the arterial pulsations cease entirely and the pressure falls virtually to zero during the responses to the shocks; the arterial pulsations are resumed with the resumption of normal ventricular beats. In this record the rate of propagation of the wave of excitation indicated by the duration of the downstroke of the response to a shock may be compared with its duration in the normal beats, indicated by the upstroke of the "R" variations. The rate of propagation of the wave in the artificial cycles is notably slower; the initial deflection and its return occupy practically the whole cycle.

In Fig. 1 (*c*) the responses are very similar in form to those of the preceding figure, but their rate is slightly higher, viz. 486 per minute. In this experiment it will be observed that there is a response to each successive shock, a result attained by very carefully increasing the rate of excitation. The rate of response has in this instance become a critical one, for on shortcircuiting the shocks a remarkable thing happens—the cycles do not immediately cease as in the previous examples, but two further cycles are maintained automatically. These subside and then the normal auriculo-ventricular beat is again assumed. These two automatic beats present only slightly different characteristics

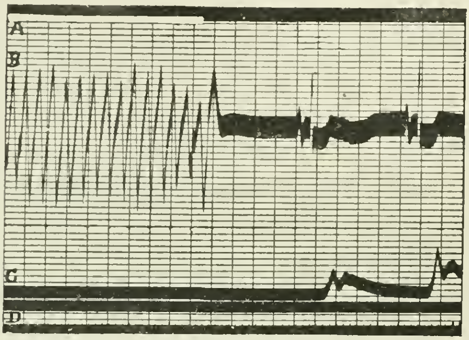


Fig. 1 *b*.

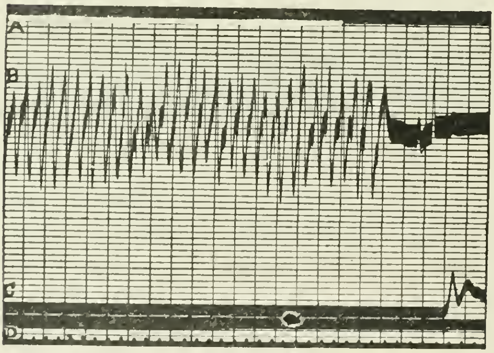


Fig. 1 *c*.

from the normal responses; the peaks are more blunted, and more especially the duration of the cycles is slightly prolonged. The three cycles preceding the final response each measure from peak to peak 2·1 mm. (=0·1235″), corresponding to the intervals between the induction shocks, but the measurement from the peak of the final response to the commencement of the first automatic deflection is 2·3 mm., and the measurement between the initial points of the two automatic deflections is 2·5 mm.

This effect may be thus interpreted—the wave of excitation has become so short and so slowly propagated that the point of excitation has become very fully excitable and capable of responding not only to an induction shock but likewise to the presumably less active excitation wave when this is allowed to reach it on cutting out the shocks. The wave of excitation reaches the point of excitation at a little later moment than that at which a following shock would be due, therefore the following cycle is a little delayed, and a reverse application of Mines' theory would possibly account for the circulating rhythm dying out as a result of this delay. It is conceivable that at a slightly more rapid rate of responses the delay would be minimised and then a permanent circulating rhythm might be initiated on cessation of the shocks.

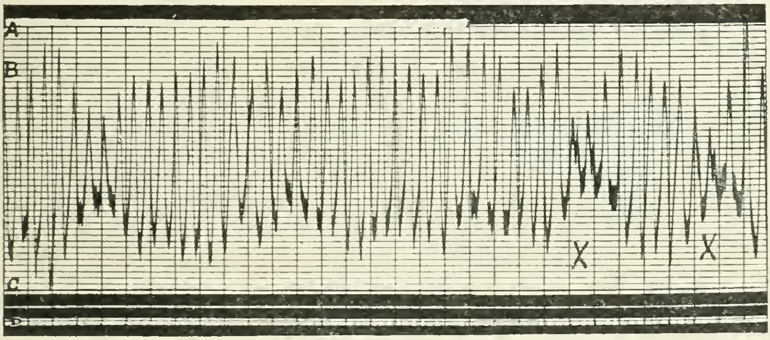


Fig. 1 *d*.

I have in one other instance succeeded in repeating this experiment, the record being almost precisely similar to that reproduced; these two automatic cycles represent I believe the earliest expression of ventricular fibrillation.

Fig. 1 *d* shows fully established fibrillation, obtained by a somewhat prolonged application of shocks at the rate of 925 per minute. There is a notable resemblance between the cycles of ventricular fibrillation and those of the responses in Figs. 1 *b* and 1 *c*; in fact the main definite difference lies in the variability of the rhythm in fibrillation, which fluctuates between 500 and 470 per minute in this instance. Further in the short periods marked × × the cycles deviate from the general type; the electrocardiogram of ventricular fibrillation always displays to a greater or less degree alternating phases of cycles of the simple type which predominate in Fig. 1 *d*, and phases of cycles of a more complicated type (Figs. 2 and 4) which approximate to those marked × × (Levy and Lewis[1]). The more irregular cycles do not appear at the moment subject to any positive explanation; but the simpler cycles usually constitute the earliest phase of fibrillation and with regard to them we are able to make the following statements: (1) They are akin in form to the cycles of a series of rapid responses to induction shocks. (2) They are in a direct sequence to such responses (Fig. 1 *c*, and Figs. 2 and 3). It may therefore be taken that they represent a recurrent process (although one of somewhat irregular rhythm), and one similar in nature to that produced by rapid successive stimuli applied to the ventricles; that this process is a circulating rhythm is the only acceptable explanation at the present moment[2]. Furthermore we may conclude that the onset of ventricular fibrillation is essentially dependent upon the rate of the preceding ventricular responses.

A sequence of responses at a relatively low rate may be maintained apparently indefinitely without causing fibrillation; thus I have recorded a sequence of responses at a rate of 330 per minute for a period of 8 minutes. A sequence of responses at a rate of over 400 per minute will generally culminate in fibrillation, though by no means invariably. The reason for the transition into fibrillation is not quite clear in such instances.

When every shock elicits a response and the rate of excitation is very gradually increased, the ventricles eventually fibrillate. There would appear to be a maximum rate of response for individual hearts, generally lying between 450 and 600 per minute; if an attempt be made to force the pace beyond this maximum, the ventricles fibrillate, or else they slow down with a 2 to 1 response, especially if the increase of rate be not sufficiently gradual. It is evident that, presuming a maximum ventricular rate to be attained, further shortening the intervals between the shocks would cause an excitation to fall within a refractory period, so that in effect the shock would be cut out and a circulating rhythm set up in consequence; this is a possible explanation of the transition under such conditions. The continuation of the shocks for some little time after the onset of fibrillation appears to have an influence in making it a permanent condition.

---

[1] *Heart*, III. 99. 1911.

[2] Further evidence in support of this conclusion is afforded in the case of ventricular fibrillation induced by the intravenous injection of a solution of barium chloride. In such a case the electrocardiogram does not differ from the usual type, but the cycles recur at a considerably slower rate, about 250 per minute, and the successive waves of contraction are perceptible to the eye, passing in rapid sequence over the ventricles without effecting any coordinate contraction.

In many of my experiments the rate of excitation exhibited spontaneous variations owing to the defect in the apparatus already referred to, and in several records a variation synchronized with the onset of ventricular fibrillation; this variation might very well cause the shocks to become ineffectual through falling in refractory periods, and in some cases in this way to be the immediate cause of fibrillation, but perfectly constructed apparatus would be essential in order to decide this point. It may be said that certain fast rates of ventricular responses tend to pass into fibrillation, but for the moment the exact conditioning cause of the transition must remain open to question.

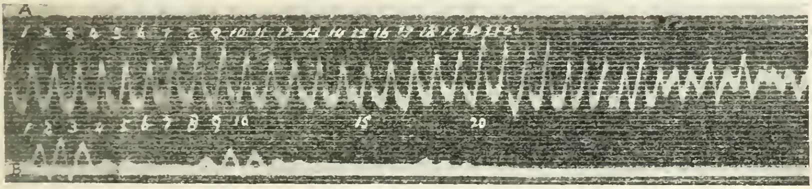


Fig. 2. × ⅔. *A*, shock signals. *B*, Hürthle curve. The upper row of figures denotes the numerical order of the shocks referred to in the text. The lower row of figures refers to the upstrokes of the corresponding electric responses.

Exp. 3 illustrates the passage into ventricular fibrillation as a result of the gradual increase in the rate of induction shocks; the record is reproduced in Fig. 2.

Exp. 3. Chloroform, 6 %. Atropine sulphate 3 mgrms. by intravenous injection. Electrodes on right ventricle; 5 mm. below the root of the pulmonary artery and 5 mm. to right of septum. The initial electric response to the shock is an upstroke in the electrocardiogram. The rate of excitation was gradually but somewhat rapidly increased; thus the interval between shocks 1 to 2=4·9 mm. in measurement, =0·14″ duration, =rate of 428 per minute. The iuterval between shocks 22–23=3·95 mm., =1128″, =rate of 531 per minute.

Each shock evokes a ventricular response, and up to the 19th shock the intervals between the upstrokes of the electrical responses are approximately equal to the varying intervals between the shocks. There is some difficulty in clearly defining the exact commencement of the upstroke, and some allowance must be made for this. The 19th cycle however is of slightly longer duration than the 19–20 shock interval, and the succeeding cycles are clearly not responses to the shocks as the discrepancy between the intervals is very marked.

Numerical index of shocks and responses	Rate of shocks per minute	Measurement between shocks in mm.	Measurement between upstrokes in mm.
10 to 11	433	4·85	5·0
11 to 12	424	4·95	5·0
12 to 13	428	4·9	5·0
13 to 14	433	4·85	4·9
14 to 15	433	4·85	4·7
15 to 16	446	4·7	4·85
16 to 17	446	4·7	4·75
17 to 18	460	4·55	4·5
18 to 19	460	4·55	4·5
19 to 20	487	4·3	4·5
20 to 21	500	4·2	4·85
21 to 22	506	4·15	4·0
22 to 23	531	3·95	4·5
23 to 24	537	3·9	4·5
24 to 25	531	3·95	3·2

It would appear that the 19th cycle is the last true response to excitation and that thereafter the cycles are automatic and that they represent in fact the condition of fibrillation. Some further evidence is afforded by the pulse curve, which is indicated by small waves up to the 19th cycle; thereafter it ceases entirely. The first few automatic beats are similar to the preceding responsive cycles and from their form alone it would be difficult to say at which point fibrillation commenced. The later cycles assume a more irregular and complicated form, and in the continuation of the record the phases of simple and complicated cycles alternate in the usual way.

In the case of faradic currents of greater frequency the sequence of events appears to be essentially the same, a succession of ventricular responses occurring at regular intervals as a prelude to ventricular fibrillation. Sometimes a rapid rate of responses is abruptly attained, as for example in one experiment in which the rate of shocks was 3000 per minute; two initial responses occurred at an interval of 7 shocks, a sequence followed of some 22 responses to every sixth shock, and then fibrillation occurred. In other cases the responses occur at successively shorter intervals and then pass into fibrillation. With rapid faradic shocks ventricular fibrillation may be delayed, but on the other hand it may occur within a very short period, *e.g.* less than one second, from the moment of application, and under such circumstances the initial phase of fibrillation may be the more complicated form, so that the electrocardiograms are not always easy to decipher, but the essential fact is that fibrillation is always preceded by a succession of responses, however short. A very rapid succession of shocks would appear to favour fibrillation in that it facilitates the adoption of a rapid rate of responses. The strength of the faradic current likewise to some extent favours fibrillation, and I believe for the same reason, but I have not sufficient experimental data to speak with certainty on this point.

Fig. 3 illustrates the whole sequence from the moment of the application of a rapid faradic current of 1200 shocks per minute. The cat was under urethane and ether. The electrodes were applied to the left ventricle. The initial electrical deflection is in a downward direction. A response of 1 in 3 was quickly assumed = 400 per minute. At the mark † the faradic rate increased spontaneously to 1300, making the responses 433 per minute and the ventricles fibrillated shortly after.

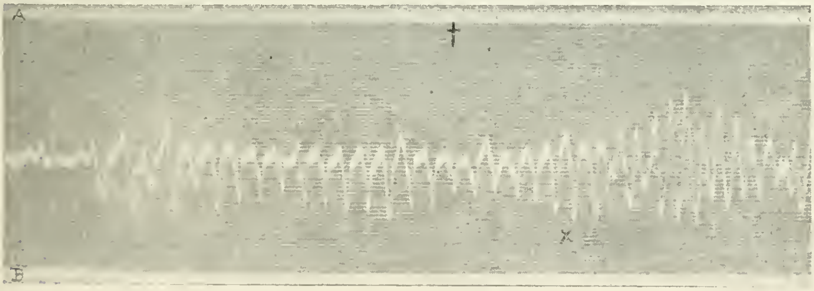


Fig. 3. × ½. *A*, shock signals. *B*, signal indicating the moment of throwing in the shocks. The last normal cycle which precedes this moment is shown.

Fibrillation apparently commences at the cycle marked ×, which cannot be identified as a response[1].

(*c*) *Fibrillation occurring in sequence to ventricular extrasystoles of intrinsic origin.* Ventricular fibrillation may be induced with considerable precision in an animal which is in a light stage of chloroform anæsthesia, by the injection of a small dose of adrenalin into a vein. Fibrillation under such conditions is never conditioned by a transition from a normal heart beat, but is always preceded by abnormalities of ventricular action of a definite kind[1]. In this antecedent stage, which constitutes the adrenalin reaction, the ventricles exhibit a succession of extrasystoles, and these are of multiple types, arising from several distinct foci; rarely they arise only from two points alternately active (Fig. 4). These extrasystoles may occur in rapid sequence, generally attaining a maximum rate of about 300 per minute, never, so far as my experience goes, exceeding a rate of 330 per minute. The condition of multiple ventricular extrasystoles thus induced very frequently terminates in fibrillation. The electrical features of ventricular fibrillation as a result of the injection of adrenalin under chloroform are precisely similar to those of fibrillation induced by faradic stimuli, in fact there can be no doubt we have to deal with precisely the same phenomenon. But the mechanism of the transition into fibrillation is obviously a different one (Fig. 4) for in this case (and in all similar experiments) the onset of fibrillation is exceedingly abrupt, and the distinction between the cycles of the preceding extrasystoles and the oscillations of fibrillation is a marked one. The rate of the cycles is furthermore abruptly raised from about 300 per minute to 500 or more per minute.

---

[1] The cycles in this record are somewhat irregular in form and their intervals cannot therefore be measured with precision.

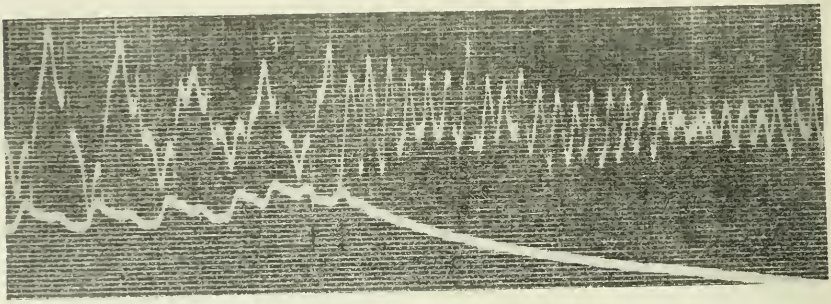


Fig. 4. × $\frac{2}{3}$. Adrenalin-chloroform reaction. Electrocardiographic and Hürthle curves. The arrows indicate the corresponding last ventricular extrasystole and last pulse wave.

In Fig. 4 alternate beats arise from the right and left ventricles respectively at a combined rate of 325 per minute. The abrupt nature of the change into fibrillation is further indicated in this figure by the sudden cessation of the pulse waves without previous lowering of the blood-pressure; the blood-pressure falls suddenly at a point coincident with the onset of fibrillation.

---

[1] Levy and Lewis, *loc. cit.*

Prof. Mines informs me he has in progress further observations upon the origin of circulating rhythms, and possibly these may throw some light upon the cause of the fibrillation in this experiment, but it does not appear to me that any extant theory affords a satisfactory explanation of its onset in this connection. I would especially emphasise the point that chloroform does not appear to favour fibrillation directly, at least it does not do so in experiments with faradic excitation; it merely favours the onset of the causative extrasystolic tachycardia.

The experimental production of ventricular extrasystoles from more than one point by means of faradic shocks throws little light on the subject; at most such experiments tend to show that there is no greatly increased tendency to pass into fibrillation when more than one point is excited. Fig. 5 illustrates a record from such an experiment. The two pairs of electrodes were applied to the right and left ventricles respectively, which were excited alternately. The electrocardiogram obtained resembles that shown in the adrenalin experiment (Fig. 4). The maximum rate obtained was 320 responses per minute; above that rate one or other of the series of stimuli failed to induce a response and the following responses were from one side only. In one experiment I maintained a rate of 290 to 300 responses per minute continuously for several minutes, the heart resuming its normal beat on cessation of the shocks. The maximum rate observed is suggestively akin to that of the adrenalin tachycardias, but I would not care to dwell on this point in default of more refined methods of observation.

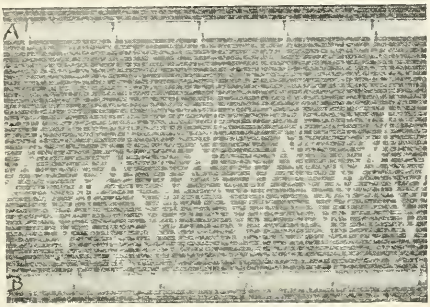


Fig. 5. × $\frac{3}{4}$. *A*, shock signals, right ventricle. *B*, shock signals, left ventricle. The *B* shocks occur very nearly, but not absolutely, half-way between the *A* shocks.

SUMMARY.

It has been shown in the mammalian heart that:

(1) A loss of ventricular function accompanies a rapid series of ventricular responses to induction shocks; this observation is subject to the explanation that the ventricles are not uniformly contracted at any one moment, and that their walls are traversed by a short

and slowly propagated wave of contraction in response to the shocks. Total loss of ventricular function is likewise a feature of ventricular fibrillation.

(2) In a heart under conditions of maximum or nearly maximum response to induction shocks, automatic cycles appear on cessation of the shocks. This is subject to the explanation that the waves of excitation pass on and themselves excite fresh cycles. The automatic cycles are in fact a rate effect.

(3) The automatic cycles resemble in electrocardiographic expression the preceding responses on the one hand, and the cycles of one phase (usually the earlier phase) of ventricular fibrillation on the other; it is inferred therefore that they are the expression of a similar mechanism, and this inference holds good whether this mechanism be that suggested by Mines or some other.

(4) The rate of the initial cycles of ventricular fibrillation approximates to that of the preceding responses to induction shocks. This again points to a similarity of mechanism.

(5) Excitation of the ventricles by the ordinary faradizing current induces ventricular fibrillation through the medium of preceding ventricular responses.

(6) The irregular phase of the electrocardiogram of ventricular fibrillation requires explanation. It is possible that ventricular fibrillation involves more than a single process.

(7) Chloroform does not appear to favour the onset of ventricular fibrillation as a result of faradization.

(8) The electrocardiogram of ventricular fibrillation in response to a series of multiple ventricular extrasystoles of intrinsic origin (*e.g.* as a result of the chloroform-adrenalin reaction) is identical with that resulting from faradization of the ventricles; the sudden transition in such a case from one state to the other awaits a satisfactory explanation.

The general trend of these observations is to support the view suggested by Mines that a circulating rhythm underlies the conditions known as ventricular fibrillation. I recognise that other views demand full consideration, but these I have refrained from discussing as it appears to me that a more ready explanation of the particular experiments described is forthcoming on the lines suggested by Mines.

I am indebted to Dr T. Lewis for kindly placing the working facilities of his department at my disposal.

NOTES OF FURTHER OBSERVATIONS UPON DYSPNOEA AND ITS RELATION TO BLOOD REACTION

By THOMAS LEWIS[1]
(Cardiographic Department, University College Hospital Medical School)

AND

JOSEPH BARCROFT
(Physiological Laboratory, Cambridge)

A Special Symptom-complex.

In a recent communication (1) we have drawn attention to a peculiar symptom-complex associated with reduced alkalinity of the blood occurring in elderly subjects, and comprising:—continuous dyspnoea, often intensified for short periods, especially at night; good or fair blood aeration as judged by the absence of cyanosis, or such cyanosis as would seem compatible with simple cardiac dyspnoea, and by examination of the alveolar air; Cheyne-Stokes breathing with or without full apnoeic periods; an increase of pulse-rate (80–100 per minute); and, lastly, a subnormal temperature. It has also been emphasized that this symptom-complex, associated as it is with cardiac enlargement, general arterial disease, and fibrosis of the kidney in greater or less degree, may be, and frequently is, combined with any of those symptoms spoken of at the present time as uraemic, such for example as temporary hemiplegia or aphasia, vomiting, headache, convulsive seizures or coma; that it may be associated with any form of valvular disease or perverted heart mechanism, and that signs of heart failure are rather the rule than the exception. The view which we have taken is that none of these associations are necessary associations any more than is the high blood pressure or the wasting which are frequent phenomena in such cases, but that they are superadded to a complex which may occur in an almost pure form. We have also drawn attention to the fact that the complex is of extremely frequent occurrence, but that more often than not it is combined with heart failure which veils the condition in greater or lesser degree. Thus considerable cyanosis may be present and the disproportion between the hyperpnoea and lack of blood aeration, the most important clinical sign, may not be distinct. It has been suggested too that in certain of the cases

---

[1] The expenses connected with these observations have been defrayed partially by a Graham Research Grant, and partially by a grant from the Royal Society.

[Q. J. M., Jan., 1915.]

where Cheyne-Stokes breathing does not appear, the excess of CO_2 in the arterial blood circulating through the respiratory centre may account for its abolition (2). The usual qualities of the urine—low specific gravity, pale colour, increased quantity, which are seen in the less complicated cases—are lost when there is cardio-vascular stasis, and a cardiac urine of high specific gravity, dark in colour, and loaded with urates and phosphates is usually passed in small quantities in its stead. Granular casts and albumin are almost always found. If too the patient is affected by inflammations, and these are by no means uncommon as terminal events, then the temperature is elevated to normal or above the line of normality. A temperature of 98·6° in such patients must be regarded as an evidence of a febrile tendency.

We have emphasized these features because the patients who suffer from acidosis present very diverse clinical pictures, and as a consequence the symptom-complex may not be easy to recognize except in the comparatively rare frank cases, unless the influence of such complications upon the symptom-complex is clearly borne in mind.

Those dyspnoeic patients who are sufferers from a reduced alkalinity of the blood, and in whom the dyspnoea is wholly or largely due to this altered reaction, are placed in different clinical categories, according as some more or less prominent system group or sign impresses itself upon the observer. Thus they pass as cases of renal disease, granular kidney, uraemia, mitral regurgitation, mitral stenosis, aneurism, aortic disease, arterial disease, emphysema, bronchitis, pleurisy, and what not.

In the present series of cases we have not studied the actual reaction of the blood, but the blood 'acidosis', i. e. the excessive acid, exclusive of CO_2, in the blood as compared with the bases present. Our test, as formerly, was the percentage saturation of the blood with oxygen when exposed at 37° C. to 17 mm. pressure of that gas. For details of the method the reader is referred to our former paper (1) or to Appendix I in *The Respiratory Function of the Blood* (5). The relation between the degree of acidosis and the percentage saturation is tabulated for the observed cases at the end of this paper; here we need only say that the percentage saturation of normal blood, when exposed to 17 mm. pressure, is 75–80 per cent. In proportion as the quantity of acids, relative to bases, is excessive the percentage saturation drops.

We report at the present time four new cases in which the symptomatology is characteristic and relatively uncomplicated (Cases 1–4), and two cases complicated by aortic disease—in one an aneurysm (Cases 5 and 6).

Case 1. *Dyspnoea in a man who presented signs of renal and myocardial affections. Nocturnal attacks. Acidosis was found on two occasions.*

A. A., a coster of 53 years, was admitted to hospital on account of shortness of breath, palpitation, and aching pain over the praecordium.

History. (8. 11. 13.) He knew of no past illness, with the exception of abscesses in the groin twelve months before. Alcohol had been taken in moderation.

Present illness. Nine months before admission he began to suffer from breathlessness upon exertion. Very soon the condition became worse and his suffering was intensified by attacks of breathlessness at night, waking him from sleep with a feeling of suffocation and lasting from 10 to 30 minutes. Usually there were one or two such attacks each night. Shortly before admission breathlessness became continuous and palpitation and pain over the praecordium were added.

On admission. (8. 11. 13.) The patient, a spare individual, was orthopnoeic and had slight cyanosis of the lips, tongue, cheeks, and ears and fingers (haemoglobin 98 per cent.). The temperature lay usually at 98°, falling often to 97°. His breathing was hurried, 44 per minute, but being shallow, was accompanied by little distress. It had a tendency to periodicity of the Cheyne-Stokes type. He could hold his breath some nine or more seconds; thus showing some reserve. The *heart's* dullness lay 1½ and 4¾ inches from the mid-line and the third rib. The apex beat was not perceptible; the heart sounds were natural but a little fast; the pulse-rate varied between 98 and 128 per minute from time to time. The mechanism of the heart was normal. The arteries were thickened, the veins were full and freely pulsating. The liver presented some enlargement. Dropsy of the legs and sacrum was present in slight degree. The chest was rigid. There were signs of hydrothorax on the left side, and signs of emphysema and bronchitis over the right chest. The *urine*, of which some 1,100–1,300 c.c. were voided daily, was of low sp. gr., and contained a faint trace of albumin and many granular casts. An examination of the blood exposed to 18 mm. oxygen pressure gave *saturations of* 66 *per cent. and* 67 *per cent.*

(28. 11. 13.) Seen three weeks later, Cheyne-Stokes breathing had definitely developed, and the nocturnal attacks were a little more frequent. Otherwise his condition remained unaltered. The blood when again tested gave a *saturation of* 66 *per cent.*

Case 2. A case of cardio-vascular affection. Dyspnoea on exertion and nocturnal angina pectoris. Acidosis found.

J. L., a caretaker of 47 years, was admitted on 16. 6. 13, complaining of breathlessness, bleeding from the nose, and pain across the chest.

History. At 19 he had rheumatic fever, at 27 syphilis, and at 45 pleurisy.

Present illness. Two months before admission he experienced breathlessness on exertion which increased and became practically continuous, with nocturnal exacerbations which wakened him from sleep. The pain had been severe, coming on from time to time in sudden attacks and shooting across his chest. Shakiness was frequently experienced. Micturition had been frequent, inconveniencing him at night.

Condition. (16. 6. 13.) The patient was a little breathless sitting up in bed or lying on high pillows; the respiratory rate was 24 per minute, the pulse 76–100. Temperature usually one degree below normal. He had no cyanosis. The heart's dullness did not appear to be increased; the heart's mechanism and sounds were normal. The blood pressure was variable between 138–200 mm. Hg. Over the lungs universal rhonchi were heard. The urine was increased in quantity; sp. gr. 1,020–30; no pus, blood or albumin. There was no oedema or signs of venous stasis (Hb. 94 per cent.).

Course. The course was slightly towards improvement on 30. 6. 13. Respirations irregular, rate 16 to 19; orthopnoea slight, very little reserve, night attacks still present from time to time. Cheyne-Stokes breathing was not seen. He had very slight cyanosis of the lips but none of the tongue and fingers. The blood chlorides were increased (Dr. Cotton) to 6·07 grm. per litre, indicating some retention; the blood area was estimated at 0·35 grm. per litre. Exposed to 17 mm. oxygen at 37° C. the blood gave a *saturation of* 61 *per cent.*

(16. 5. 14.) The patient has since been attending the Out-patient Department and, passing a life of inactivity, his condition has remained unchanged.

Case 3. A case of arterio-sclerosis and renal affection in a woman who exhibited breathlessness. Nocturnal attacks and Cheyne-Stokes breathing present. Acidosis found.

Mrs. G. (26. 11. 13.) This patient, a woman of 62, was seen on a single occasion. There was no history of past illness but of dyspnoea with frequent nocturnal attacks and intermittent swelling of the legs for three years.

Condition. The heart was not materially enlarged, there were no murmurs. The mechanism was normal; rate 96–112 per minute. The arteries were tortuous and thickened; S.B.P. 160 mm. Hg. The veins were full, the liver large. Dropsy of the legs was considerable. There was slight cyanosis of lips, tongue, and fingers (Hb. 108 per cent.). The respirations were rapid and distressed (42 per minute) during the hyperpnoeic period, which lasted 7 seconds. The apnoeic period was of similar duration. The urine was abundant and contained albumin and abundant granular and hyaline casts. The temperature varied between 98° and 99°. The blood gave a *saturation of* 64 *per cent. to* 67 *per cent.* when exposed to 17 mm. oxygen pressure.

Case 4. A case of cardio-renal affection in an old woman. Cheyne-Stokes breathing and dyspnoea prominent.

Mrs. F., a wasted woman of 77 years, was seen on a single occasion (3. 12. 13). There was a six months' history of breathlessness and no past history of other illness. The patient was orthopnoeic, presented very slight cyanosis of ears, lips, and tongue (haemoglobin 100 per cent.). The temperature was 97°. The pupils were pin-point, reacting sluggishly to light, and the knee-jerks were not obtainable. The *heart's* dullness was increased, a systolic apical murmur was present, the auricles were fibrillating (ventricular rate 88 to 112 per minute). The arteries were considerably thickened; S.B.P. 130 mm. Hg. The respirations were moderately deep. rate 30–50 per minute and periodic (hyperpnoeic period 67 seconds, apnoeic period 26 seconds). There was not a great deal of distress; the breath could be held for a few seconds during the hyperpnoeic period. The chest was rigid and a few bronchitic signs were present. The veins were distended and showed pulsation; the liver was distinctly enlarged; slight oedema was present over the shins.

The blood gave *saturations of* 55 *per cent. and* 56 *per cent.*

Case 5. Aortic disease and aneurysm, cardiac failure, renal involvement. Dyspnoea, often intensified for short periods, was a chief feature of the case. Cheyne-Stokes breathing was found.

T. E., a carpenter of 44 years, was admitted to hospital on 19. 9. 13 complaining of shortness of breath, swelling of the feet, and palpitation.

History. (19. 9. 13.) At 19 years he acquired syphilis; at 30 years 'Mediterranean Fever'.

Present illness. For 5 years he had suffered from shortness of breath, most conspicuous on exertion, and more recently constant and occurring in intensified paroxysms which wakened him often each night. The sleep was very broken.

On admission. (19. 9. 13.) The patient was orthopnoeic, the respirations rapid (28 per minute); the breathing laboured and periodic (Cheyne-Stokes type). The face was pallid (haemoglobin 98 per cent.). Temperature 96–98°. The arteries were thickened and tortuous; the S.B.P. varied between 150–170 mm. Hg; water-hammer pulse. The *heart's* mechanism was normal; rate 76–108 per minute. The visible pulsation and area of dullness were notably increased. A systolic murmur and gallop rhythm were audible at the apex, and a coarse to and fro murmur at the aortic cartilage was heard. There was a large area of dullness over the manubrium and upper ribs, and a skiagram showed the outline of a large pulsating tumour. The veins were engorged; the liver was greatly enlarged, ascites was present and a right pleural effusion. The legs and lower trunk were dropsical. The *urine*, sp. gr. 1,025; cloud of albumin; granular casts; quantity 150–3,150 c.c. per diem.

Course. On 1. 10. 13, four pints of fluid were removed from the chest and he obtained a little relief. On 2. 10. 13, the condition showed some improvement; the dropsy had declined. The respiratory rate was 36 per minute during the hyperpnoeic periods; these lasted each about 50 seconds and were followed by apnoeic periods of 10 to 20 seconds. Distress was still considerable. Very slight cyanosis was seen in the lips, tongue, ears, and fingers. The veins and liver were engorged as before, and there were general bronchitic signs. Examination of the blood gave 60 *per cent. saturation.* Later the oedema increased, necessitating drainage, and vomiting appeared; the patient sank gradually and died on 14. 11. 13.

Post-mortem. The body showed considerable anasarca and fluid in all the cavities. The heart weighed 877 grm. (vent. muscle, 563 grm.; R. = 135, L. = 362, and septum = 66). The aortic valves were crumpled and incompetent. The cavities were dilated. the coronaries patent and wide but very calcareous. A large sacculated aneurysm was found upon the arch of the aorta. The liver was of nutmeg type, the spleen firm and congested. The *kidneys* weighed 170 grammes apiece; they were tough and congested, but the surfaces were smooth and the capsules stripped easily. In the *lungs* patches of collapse were found.

Case 6. A case of aortic disease, cardiac failure. and renal involvement. Dyspnoea, Cheyne-Stokes breathing. nocturnal intensification of breathlessness, prominent. Terminal pneumonia present.

G. G., a caretaker of 42 years, admitted to hospital complaining of shortness of breath, pain in left shoulder and chest.

History. He had suffered from rheumatic fever and pneumonia at 18 years.

Present illness. (18. 11. 13.) Shortness of breath, especially upon exertion, had been present for two years. At night he was often awakened, and sometimes repeatedly, by severe breathlessness, lasting a quarter to half an hour. Before admission the breathlessness and pain had been continual.

On admission. (18. 11. 13.) The patient was orthopnoeic, the breathing laboured (rate 40 per minute). Cheyne-Stokes breathing was present (hyperpnoeic period 40 seconds, apnoeic 10 seconds). His face was pallid (haemoglobin 78 per cent.). He had slight cyanosis of cheeks and ears, lips, and tongue. The breath had a sweet ethereal smell. The temperature was 100·8°. The heart's action was rapid (pulse 128 per minute); its mechanism normal; the area of dullness and pulsation being greatly increased; a systolic murmur was audible at the apex, and rough to and fro murmurs at the aortic area. The arteries were thickened; S. B. P. 126 mm. Hg; water-hammer pulse. Veins full and pulsating. The liver greatly distended. No oedema or ascites. The *urine* was decreased in quantity (250–1,100 c.c.) and of high colour; sp. gr. 1,030, and contained a cloud of albumin and granular casts. The *lungs*: there was profuse sputum, which was blood-stained. The chest was hyper-resonant; the breath sounds harsh. A little impairment of note was discovered at the right apex; râles and rhonchi were universal.

A positive Wassermann reaction was discovered. The blood exposed to oxygen gave *saturations of* 46 *per cent. and* 52 *per cent.*

Course. The temperature fluctuated between 97·5° and 102°; he became weaker and delirious, dying on the 22nd.

Post-mortem. Fluid was found in both pleurae, the bases of the *lungs* were in a state of red hepatization. The *heart* weighed 632 grm. (muscle, 416·5 grm.; R. 129, L. 241, and septum 46·4 grm.). The aortic valves were crumpled and retracted, regurgitation having been free. The mitral and tricuspid rings and the heart chambers were dilated. The orifices of the coronary vessels were a little narrow, but the vessels were healthy. The *kidneys* weighed 242 grm. apiece; they were congested; the capsules a little thickened. The epithelium was swollen and granular; little or no fibrosis; intimal thickening of the vessels. The liver was enlarged and nutmeg. The spleen was swollen and its capsule thickened.

Simple Cardiac Dyspnoea.

In our first report we gave the details of examination in three cases of heart disease in which there was no acidosis, and in which the breathlessness could be attributed solely to deficient aeration of the blood. At the present time, we add three similar cases to the list (Cases 7, 8, and 9) of those in which no acidosis was discovered.

Case 7. Cardiac failure of rheumatic or specific origin, in a man who exhibited mitral stenosis and aortic disease. Dyspnoea present. No acidosis.

G. W., a man of 35 years, was admitted with symptoms and signs of cardiac failure on 10. 10. 13.

History. Syphilis at 21 years; rheumatic fever at 23 years of age.

Present illness. Shortness of breath on exertion or at rest, and swelling of legs, of one year's duration, frequent headaches and sleeplessness were the chief symptoms.

Condition. (11. 10. 13.) An orthopnoeic patient of high colour (Hb. 104 per cent.) who presented some cyanosis. The breathing was shallow and 28 per minute. The veins and liver were engorged. No ascites, but moderate dropsy of legs and signs of oedema at lung bases. The heart enlarged, free aortic regurgitation; a full diastolic rumble at the apex and blowing systolic murmur. Auricular fibrillation (heart rate 100). Temperature 97–98°. Blood pressure 96–100 mm. Hg. *Urine* dark, sp. gr. 1,030, scanty, containing urates and albumin.

Course. (2. 11. 13.) He could lie flat without much discomfort, but the breathing became increased in rate. While propped up the rate was 28 and regular; there was no distress; he had some reserve, the breath being held 15 seconds, but it was incomplete. He had had attacks of nocturnal breathlessness but was at the time free from them. There was slight cyanosis of lips and very slight of ears and tongue. The engorgement of veins and liver had gone, as had the dropsy. The blood exposed to 17 mm. oxygen pressure gave a *saturation of 77 per cent.*

Case 8. A case of mitral stenosis with auricular fibrillation and cardiac failure. No acidosis found.

Mrs. S., a woman of 28 years, was admitted on 10. 6. 13, complaining of pain in the abdomen and shortness of breath.

History. Rheumatic fever at 20 years.

Present illness. Pain had been present over the upper abdomen and she had had shortness of breath for two years. The symptoms had recently become more urgent. Colour high (haemoglobin 90 per cent.).

Condition. (11. 6. 13.) The patient was orthopnoeic, but while sitting there was but little dyspnoea (rate 23 per minute, respirations irregular). A little cyanosis. The heart was enlarged and showed clear signs of mitral stenosis and fibrillation. Arteries normal; S. B. P. 126–136 mm. Hg. The liver considerably enlarged. The veins full. No ascites or oedema. Spleen palpable. The *urine* contained a trace of albumin; quantity 400–1,350 c.c. per diem; sp. gr. 1,030–1,019. Temperature normal.

Course. (30. 6. 13.) Orthopnoea still present. A little dyspnoea (rate 20–24, a little laboured); she had a good deal of reserve, holding the breath 20 seconds, but it was by no means complete. Slight cyanosis present in lips, tongue, cheeks, and ears. Blood chlorides 5·74 and urea 0·33 grm. per litre (Dr. Cotton). The blood exposed to 17 mm. oxygen pressure gave a *saturation of 74 per cent.*

Case 9. Cardiac failure in a patient who exhibited mitral stenosis. Dyspnoea present, but no acidosis.

Mrs. A., a woman of 26, was admitted to hospital complaining of breathlessness and swelling of the legs.

History. There was no history of past illness, with the exception of bronchitis, developed sixteen months before and leading up to the illness for which she was admitted.

Condition. (2. 11. 13.) Seen on a single occasion, she was orthopnoeic, and gave a history of nocturnal attacks of breathlessness, the result of slipping down the bed off her pillows. She was flushed (Hb. 90 per cent.) and showed slight cyanosis of lips and cheeks, but none of the ears and fingers. Respirations were at 25 per minute, regular and deep but not distressed; of reserve there was little or none. The *heart* was enlarged, and clear signs of mitral stenosis were present; its mechanism was normal, the pulse being 108–128. The arteries seemed normal; S. B. P. 100 mm. Hg. The veins were a little full but did not pulsate freely; the liver showed conspicuous enlargement. She had dropsy of the legs and body wall. The urine varied in quantity from 390 to 1,300 c.c. per diem, and contained a trace of albumin and hyaline casts. Over the lungs there were universal signs of bronchitis. The temperature lay between 97° and 98° as a rule. The blood exposed to oxygen gave a *saturation of 77 per cent.*

Speaking with these cases and those of our previous report in view, it seems to us that from the point of view of blood reaction and dyspnoea, cases of heart disease may be divided into two classes. There are the relatively simple and uncomplicated cases, where, though failure may be present originally, recovery is the rule. These are usually young subjects with fresh or heightened colour, and although cyanosis, slight, moderate, or deep, is present, dyspnoea, as indicated by the rate of respiration, is not great. The respirations range from 25 to 35 per minute, orthopnoea is present, and the field of respiratory response is strictly limited. In these patients no acidosis is found.

Young patients affected by mitral stenosis do not present reduced blood alkalinity in our experience, unless the condition is complicated. It is quite apparent from our observations that acidosis is a frequent accompaniment of heart failure, especially in middle-aged or elderly subjects, and *a fortiori* in chronic or terminal heart failure. But that heart failure with signs of venous engorgement and oedema of the legs may occur apart from this intoxication is equally clear. The acidosis cannot be attributed to heart failure alone, and we turn naturally to the excretory organs in seeking its explanation.

The prevalence of reduced alkalinity of the blood in heart patients may be judged from the statement that the majority of those patients who are persistently distressed for want of breath, and who, with muddy and sunken countenances, sit in our wards and exhibit the classical signs of heart failure, be they patients who gradually sink and die, or be they patients who are temporarily relieved, are sufferers from the condition considered.

The Renal Factor.

It has been stated in our previous report that those cases of heart disease which have shown acidosis have also presented signs of renal involvement, clinical or *post mortem.* The extent to which the acidosis may be attributed to a renal factor has been discussed by us, and we have avoided a final decision, as in our reports we have also avoided the use of the term uraemic. Although many of our patients would unquestionably be termed by many observers uraemic or uraemic asthmatics, the application of such terms to them is evidently fraught with danger. The terms presuppose the origin of the dyspnoea in a renal lesion; ultimately it may or may not be found to result from such.

To tabulate patients in whom reduced alkalinity has been found under the heading of uraemia, even though Straub and Schlayer (4) and others (3) have found evidence of this condition of the blood in cases diagnosed as uraemic from other signs, is to perpetuate a suggestion for which there is neither proof nor disproof, namely, that such reduced alkalinity is of renal origin. The safer policy at the present stage of the investigation is fully to report the facts on the clinical side, leaving the ultimate solution and the final clinical grouping to a time when our observations are more complete.

That there is very suggestive evidence of a metabolic disorder of renal origin is true, and two cases which we have recently observed would seem to strongly support such a contention (Cases 10 and 11).

Case 10. *Pregnancy in a patient suffering from rheumatic heart disease. Breathlessness accompanied by acidosis.*

M. B., a woman of 23 years, was admitted to hospital on 5. 6. 14.

History. At 20 the patient had rheumatic fever.

Present illness. For seven months there had been increasing difficulty in mounting stairs, on account of breathlessness. For two months the breathing had been more difficult, preventing her sleeping except in a chair or with many pillows. Morning vomiting had distressed her for two months, and she had suffered from cough with blood-stained expectoration for the same period.

Condition. (8. 6. 14.) A cheery and well-nourished primigravida, daily expecting confinement. She was orthopnoeic; the respiratory rate was 44, but the breathing shallow. The colour was good, a little cyanosis only being present in the lips. She was able to hold the breath five seconds, but deep breathing was followed by no trace of apnoea. The temperature was usually subnormal (97–99°). The heart was somewhat enlarged; a thrill was present at the apex, and other evident signs of mitral stenosis accompanied it. The mechanism was normal but for occasional extra-systoles. The pulse-rate was 88 per minute. Oedema of the legs and sacrum was present. The veins were a little full; enlargement of the liver could not be detected. S. B. P. 108–125 mm. Hg. The *urine* varied in quantity from 150 to 1,400 c.c. per diem, being usually about 500 c.c.; sp. gr. 1,013–1,030; albumin and granular casts present. *The lungs*: beyond a little basic dullness with a few crepitations the lungs and pleurae seemed normal. The blood exposed to oxygen showed *acidosis*, but its actual degree was uncertain but considerable, corresponding to a degree indicated by something of the order of 45 per cent. saturation for an exposure of the blood to 17 mm. oxygen.

(15. 6. 14.) By this date the condition had improved. Breathlessness was less, but still present; the respirations being 32 per minute; the breath could be

held five seconds and deep breathing produced no apnoea. There was no cyanosis and the dropsy had decreased a good deal. The pulse-rate was 76. The blood exposed to oxygen gave *saturations of* 53 *per cent. and* 57 *per cent.*

(16. 6. 14.) The patient was delivered of a full-term child without accident.

(22. 6. 14.) There was much improvement. The pillows had been removed; the respiratory rate had fallen to 26; the breath could be held fifteen seconds. The colour was fresh and the patient comfortable.

The chief interest of this case of mitral stenosis is its associated pregnancy. It suggests to us that eclamptic breathlessness may be of acid origin. Other cases of the same valve affection, in which renal lesions were discovered at autopsy and in which breathlessness was largely attributable to altered blood reaction, have been recorded previously.

Case 11. *A case of large white kidney, with dropsy of renal type, complicated by aortic disease and slight mitral stenosis. Urgent breathlessness in the absence of cyanosis was a feature of the case. Acidosis was discovered.*

J. N., a man of 37 years, was admitted to hospital complaining of anaemia, swelling of the body, and breathlessness.

History. (24. 10. 13.) There was no history of past illness or of alcoholism.

Present illness. In April, 1912, the patient began to develop puffiness of the eyelids, and a little later swelling of the scrotum, arms, and legs. He had been failing in strength for some time previously. At the same time he noticed that he was becoming paler and that he could not exert himself without becoming breathless. Shortly before admission his breathlessness was more conspicuous towards and during the night, preventing him from sleeping or waking him repeatedly. He suffered from nausea but had no vomiting.

On admission. (24. 10. 13.) The patient lay propped with pillows. He was very pale (haemoglobin 50 per cent.) but exhibited no trace of cyanosis. He had universal oedema of the boggy type. The eyelids, cheeks, and conjunctivae were dropsical; the arms were enormously infiltrated with fluid; so too were the lips, genitalia, and body wall. There were signs of pleural effusions and of a collection of ascitic fluid. The temperature showed variations between 97·5° and 101°. Respirations were very laboured at a rate of 36 per minute; there was no reserve of breathing, the respirations were regular. The breathing was more hurried, so it was stated, at night, and the distress of the patient great. Cheyne-Stokes breathing was not seen at this or any other time. The *heart* presented signs of enlargement; its action was regular at 118–120 per minute. A systolic murmur was audible over the epigastrium. The blood pressure, obtained after gradually squeezing the tissues of the raised arm free of oedema, was measured at 160 mm. Hg. The brachial artery in this arm seemed thickened. There was no appreciable engorgement of the veins; the liver showed no sign of engorgement. The *urine* had a sp. gr. of 1,020, and contained abundant albumin, a good deal of blood (foggy or coloured), and granular casts; the quantity varied from 370 to 780 c.c. daily. Examination of the blood gave *saturations of* 43·5 *and* 44 *per cent.* Death occurred on the 29th of the same month.

Post-mortem. Universal oedema, great ascites, and large pleural effusions were discovered. The *aorta* was healthy, the peripheral vessels atheromatous. The *pericardium* was universally adherent by tissue which readily broke down. The *heart* weighed 747 grm., the muscle of the ventricles weighed 423·5 grm. (R. V. 112; L. V. 243; septum 68·5). The coronary arteries were normal; no clots were found in the cavities. The aortic valves were a little crumpled at their margins and slightly incompetent: at their bases they were calcareous. An old-standing abscess, a centimetre in diameter and having thick fibrous walls, was discovered between aorta and pulmonary artery immediately above the valves. The mitral valves were thickened and the orifice narrowed to 260 sq. mm. The *liver* weighed 1,760 grm. and was fatty and congested; the *spleen* weighed 277 grm.; the capsule was thick, the substance pale and friable. The *lungs* presented patches of collapse and emphysema; small infarcts were found in both. The *kidneys* were large (341 grm.) and pale. The surfaces were smooth, the capsules stripped easily. The cortex was broad, the Malpighian corpuscles prominent; the substance of the organ was firm. Histologically, the organs demonstrated a chronic parenchymatous nephritis with diffuse interstitial change. The nuclei of the Malpighian corpuscles had proliferated: no hyaline corpuscles were seen. There was a good deal of interstitial fibrosis and lymphocytosis, and some intimal thickening of the vessels.

The second case (Case 11) is of importance because it is the first of our series in which the nephritis was of a conspicuous parenchymatous type. Clinically this man appeared to be a characteristic instance of the large white kidney; the universal dropsy of frank renal type, and the character of the urine, pointed clearly to gross renal insufficiency; yet at the post-mortem unsuspected aortic and mitral lesions, inconspicuous it is true, were discovered. It seems to us that observations are still required upon instances of a still purer kind, namely, upon those in whom renal lesions are the sole gross or manifest affections, before we can finally state that dyspnoea of purely renal origin exists. That renal dyspnoea so called is the result of reduced blood alkalinity we see no reason to doubt; but that is an entirely different statement, for the term uraemic is comprehensive and is applied clinically to a variety of conditions, the exact nature of which is still unknown. We require also chemical investigation as well to give us a clear insight into the actual metabolic changes occurring in the body.

Certain it is that in the cases of acidosis which we describe we have to deal with a changed metabolism as profound in its symptomatic effects as is the altered metabolism of diabetes, and of far greater consequence than the latter, because so much commoner.

A Note upon Mechanical Dyspnoea.

To refer dyspnoea entirely and solely to mechanical causes when we have clear evidence of a lesion which is known oftentimes to obstruct the respiratory passages, or where we are certain that a large proportion of the lung tissue is out of action, or when deficiencies of the pulmonary circulation are suspected, is a temptation to which we yield only too naturally. But it appears that our reasoning is not always to be justified; we have reported, for example, an instance of aneurysm in which continuous dyspnoea, often exaggerated, might have aroused the facile explanation of obstructive dyspnoea from pressure on trachea or bronchus. That such was not the main cause is shown by the blood analysis. Our next case may be used as an even more notable example of the same point of view (Case 12).

Case 12. *Pneumothorax giving rise to acute illness in a dormant case of pulmonary tuberculosis. Dyspnoea accounted for, partially at all events, by acidosis.*

F. P., a police officer of 25 years, was admitted to hospital on 27. 4. 14 acutely ill.

History. The only past illness was a history of early tuberculosis for which the patient was treated in a sanatorium from July to December, 1913. His acute illness started the day before admission with fever and breathlessness.

Condition. (27. 4. 14.) The patient, a well-developed man, lay in bed. The skin was hot, the face flushed (Hb. 98 per cent.). He had very slight cyanosis, but a good deal of dyspnoea. The temperature was 103·4°, the pulse 128, and respirations 40 per minute. There was some mental confusion. Weak breath sounds and dullness to percussion were discovered in the left axilla. The heart limits and sounds were normal. The patient was considered to be suffering from acute pneumonia.

Course. On 29. 4. 14 the temperature remained unchanged. A slight herpetic eruption had appeared upon the chin. Breathing was less hurried. The respiratory rate at the time of examination was 24, though respirations were deep and the alae nasi were working. The colour was high; slight cyanosis being also present in the lips. A blood examination showed no acidosis, the percentage *saturation being 75 per cent.* Pneumococci were abundant in the sputum, which was frothy, but no tubercle bacillus could be found. On 1. 5. 14 the heart's dullness was first missed, and by the 7th it was perfectly evident that the patient had developed hydropneumothorax (Bell sound and subsequent radiograms). At examination on 4. 5. 14, the temperature was 101·6°, respirations 42, and the pulse 180. The patient's distress had increased, though there was little cyanosis. Blood examinations gave *saturations of 52 per cent. and 60 per cent.* On the 7th, sixteen ounces of turbid sero-fibrinous fluid were drawn from the left pleura; it contained numerous polymorph leucocytes, but no organisms (tubercle bacilli ultimately discovered in inoculated guinea-pig). From this date he improved considerably, the temperature falling gradually to normal on May 18. On this day the pulse was 78; the respiratory rate had fallen abnormally low, being 12 per minute. The patient had recovered his respiratory reserve. Blood examination gave *saturations of 77 per cent. and 79 per cent.*

In a patient who has breathlessness and clear signs of collapse of one lung, the hyperpnoea proves to be wholly or in part due to acid intoxication. Observations of this kind show only too clearly how dyspnoea of toxic origin may be interpreted erroneously upon purely physical lines, and awaken considerable distrust of mechanical explanations as they are commonly applied in individual cases.

In exophthalmic goitre breathlessness is not uncommon; where there is serious embarrassment, an enlarged thyroid or thymus gland is often held responsible. The following case (Case 13) shows that breathlessness may be due to a third cause, namely, altered blood reaction:

Case 13. *A case of exophthalmic goitre, complicated by maniacal symptoms, persistent vomiting, and distress of breathing in a young man. Acidosis was found. Death from exhaustion.*

A. L., a market stall-keeper of 30 years, was admitted complaining of pain in the pericardial region and difficulty in breathing.

History. (9. 12. 13.) With the exception of a temporary cough and blood-spitting, three years before, he knew of no illness.

Present illness. Two years before admission he had first noticed tremor of the hands and swelling and roundness of the neck. A little later, pain over the heart and difficulty in breathing were observed. Six months before admission the eyes became prominent. Headache, beating in the neck, and sleeplessness have disturbed him; the most recent symptom had been diarrhoea.

Condition. (9. 12. 13.) The patient was very dyspnoeic, respirations 46 per minute, and distressed. The colour was natural (blood counts normal); the skin moist. He had characteristic signs of exophthalmic goitre. The thyroid was enlarged uniformly, the neck pulsated freely and exhibited a systolic thrill. The hands and fingers were tremulous; the eyeballs prominent; von Graefe's sign present. The pulse was 138; the S.B.P. 128–140; the action of the heart was regular. The heart's impulse was forcible, but the dullness was not increased. The lungs presented no physical signs. *Urine* normal. There were no further signs of involvement of the nervous system.

Course. During his illness the temperature was normal. He had persistent vomiting of an intractable type and suffered greatly from sleeplessness. The breathing was not embarrassed to a constant extent, but being always difficult, it became urgently laboured in attacks lasting a few minutes or half an hour. On 15. 12. 13 the breathing was rapid and shallow, but showed some reserve; he had slight orthopnoea; forced breathing was followed by no period of apnoea but by hurried respirations. The blood exposed to oxygen gave *saturations of* 52 *per cent. and* 56 *per cent.* Alveolar air samples contained 5·4 and 4·6 per cent. CO_2 (estimation of K, 0·00018).

The patient developed maniacal symptoms, dyspnoea and vomiting continuing, and he died from exhaustion (18. 12. 13).

That the acidosis of this case was of the same nature as that discovered in cardio-renal patients seems to us improbable; we have made no further attempt to identify the actual error of metabolism, being content at present to record an alteration of blood reaction originating in the presence of a non-volatile acid.

Pneumonia.

In one of our cardio-renal cases (Case 6), pneumonic consolidation of the lungs was found at autopsy; it was considered advisable to control this case by observations upon simple pneumonic patients; more especially as the degree of dyspnoea often appears to be in excess of the cyanosis in this malady.

We include reports of four cases (Cases 14, 15, 16, and 17), in each of which a non-volatile acidosis was discovered. The acidosis of pneumonia is present during the febrile stage of the disease (Case 14) and remains for some while after the crisis, disappearing hand in hand with the breathlessness during convalescence. It may be of considerable degree (Cases 15 and 16); in two patients in whom the oxygen saturation fell to or below 40 per cent., the termination was fatal. Further observations should determine the diagnostic significance of the blood reaction in these cases, and may possibly show it to be of prognostic significance.

That acute pneumonic infection may be responsible for an altered blood reaction suggests that we should inquire further into the cause of dyspnoea in such conditions as septicaemia, infective endocarditis, &c.; for it may be that similar changes will be found in these patients also.

Case 14. *A case of acute pneumonia in a young man, ending in recovery. On the fourth day acidosis was found. The crisis occurred on the eighth day; acidosis, though of lesser degree, was present on the tenth day; during convalescence the blood reaction was normal.*

T. D., aged 27, a beer drinker, was admitted to hospital on 22. 12. 13, complaining of vomiting, headache, and pain in the right side of his chest.

History. (22. 12. 13.) He had pneumonia 7 years ago. Alcohol consumption free.

Present illness. On Saturday, the 20th, he was at work and well: on Sunday afternoon, the 21st, he felt chilled, and in the evening malaise and shivering were experienced. Pain in the right side, headache, and vomiting developed.

On admission. (22. 12. 13.) A robust man who lay a little propped in bed. The mucous membranes were of healthy colour. The breathing was hurried, 28 to 30 per minute: the temperature 98·8°. He had herpes of lips and nose. The *lungs:* the right side of the chest showed limitation of movement. dullness to percussion and prolongation of respiration. The action of the *heart* was rapid, 104 per minute, otherwise this organ seemed normal. The urine was normal.

Course. The temperature rose on the day of admission to 103·4°; it oscillated over one degree and fell gradually from a maximal of 103·4° to 100·5° on the 28th; from this point it sank abruptly to the normal and subsequently remained at or about normal for the rest of his stay.

(24. 12. 13.) On this, the fourth day of illness, an extensive patch of dullness could be made out, extending from the spine of the scapula to the base; over this area expirations were prolonged. Râles and rhonchi were audible over both bases posteriorly and friction in the axilla. The sputum was rusty. The *heart* was not enlarged (rate 106–112, regular). The S.B.P. was 128 mm. Hg. Venous reservoirs and arteries normal. *Urine*, sp. gr. 1030; contained a trace of albumin, but no casts.

The respirations were 32 per minute, he had little or no distress, the breath could be held for 15 seconds and apnoea followed deep breathing. There was no cyanosis. The blood exposed to 17 mm. oxygen pressure gave *saturations of* 59 *per cent. and* 60 *per cent.*

(28. 12. 13—8th day.) Temperature normal; bronchial or tubular breath sounds over right lung posteriorly; crepitations over both bases.

(30. 12. 13—10th day.) Temperature 97–98°, some delirium. Respiratory rate 24–28 per minute, pulse 76–80 per minute. There was no distress or cyanosis. The signs at the right base were beginning to clear up. *Saturation of blood exposed to oxygen* 67 *per cent.* From this time onwards improvement was rapid and steady, and by 14. 1. 13 the dullness at the right base had almost vanished.

(26. 1. 14). There remained a few crepitations at the right base and some increased conduction of respiratory and vocal sounds. The temperature and pulse-rate were normal. He was perfectly comfortable, the respiratory rate being 20 per minute with full reserve. *Saturation of blood exposed to oxygen* 70 *per cent. and* 72 *per cent.*

Case 15. *A case of severe acute pneumonia in a young man, terminating fatally. Profound acidosis was discovered on the tenth day of illness.*

M. G., a clerk of 22 years, was admitted to hospital on 30. 5. 14, complaining of shortness of breath and pains over the whole chest.

History. (3. 6. 14.) As a young child he had whooping-cough, measles, and pneumonia. At 11 and 12 years he had rheumatic fever and at 16 years pleurisy. He had taken no alcohol.

Present illness. His symptoms developed suddenly one week before admission and had gradually increased in severity.

On admission. (30. 5. 14.) He lay propped in bed, the cheeks flushed; the temperature being 103°. Respiration was hurried and distressed, the rate being 44 per minute. There was a little cyanosis. The *lungs*: movement of the chest wall was deficient on the left side, resonance was increased, and bronchial breath sounds were audible at both bases. The sputum was rusty and contained numerous streptococci and pneumococci. The *heart* limits were normal; an occasional soft systolic murmur was audible at the apex. The pulse was regular, the rate being 120 per minute. *Urine*, sp. gr. 1,015; contained a faint cloud of albumin.

Course. The maximal temperature fell gradually to 101° on June 7, fluctuating over two or three degrees.

(2. 6. 14.) Temperature 101·5°; the signs in the lungs were much as before. The pulse-rate was 45 per minute and breathing was laboured. He had practically no cyanosis. He was anxious and tending to delirium. *Saturation of the blood exposed to 17 mm. oxygen pressure 34 per cent.*

By June 7 it was obvious that almost the whole left lung was involved and signs of some consolidation were also present on the right side. The patient was delirious: an exploratory puncture at the left base yielded a small quantity of very blood-stained fluid. Early in the morning of the 8th large quantities of rusty sputum were ejected, the breathing being much embarrassed. He collapsed and died shortly afterwards. A post-mortem was refused.

Case 16. *A case of acute pneumonia, ending fatally, in an elderly woman. Acidosis was found on two occasions during the course of the illness. The* CO_2 *tension in exposed air was low.*

Mrs. W., a woman of 74 years, was admitted to hospital on 1. 12. 13 complaining of shortness of breath, cough, and pain in the right chest.

Present illness. The illness was of four days' duration; the onset sudden.

On admission. (3. 12. 13.) Seen on the sixth day of the disease, the patient lay in bed, propped with pillows and in some respiratory distress and delirious. There was slight cyanosis of lips, ears, cheeks, and tongue. The temperature was 102·5°. Herpes was present on the lip. The respiratory rate was 36 per minute; there was no reserve. Dullness and crepitations with friction accompanied by weak hollow breath sounds were found in the right axilla and at the base. The sputum was scanty and a little stained. The pulse-rate was 114, the heart being regular. The *heart* showed some increase in size; mitral regurgitation was present. The arteries were thickened. The blood exposed to 17 mm. oxygen pressure gave *saturations of* 49 *per cent. and* 53 *per cent.*

Course. The temperature remained high and irregular (99–103·5°) until she was seen again on 10. 12. 13. The physical signs were then found to be practically unaltered, though the patient was weaker. The respirations were still 36 per minute, though perhaps less laboured than before. The pulse-rate was 126 per minute; there was no cyanosis. The blood gave a *saturation of* 66 *per cent.* Collected samples of expired air contained 3·0 and 3·3 per cent. CO_2. The patient died on the 11th, a post-mortem investigation being refused.

Case 17. *A case of acute lobar pneumonia in an otherwise healthy young man; ending in recovery. Acidosis was found.*

W. H., a clerk of 22 years, was admitted to hospital (25. 12. 13) complaining of pain in the left chest, dizziness, sickness, and weakness of two days' duration.

History. (25. 12. 13.) Habits temperate. No past illness of consequence.

Present illness. His illness commenced acutely on the 23rd with the above-mentioned symptoms.

Condition. (25. 12. 13.) The patient lay propped in bed, was dyspnoeic and weak, the face flushed and the skin dry. The temperature 103–105°, the respirations 34, and the pulse 106 per minute. The heart showed no sign of enlargement, its action was rapid but regular, the sounds were normal. S.B.P. 111–120 per minute. The *urine* was high coloured, but otherwise normal. *Lungs*: there was limitation of movement and dullness to percussion in the left axilla and behind over the whole lower lobe; at the top of this dull area the breath sounds were harsh, below they were weak or absent. Friction was audible below the angle of the left scapula. The sputum consists of greenish sticky mucus.

Course. The crisis occurred on the evening of 27. 12. 13; the temperature falling rapidly from 103° to normal and remaining there. On 30. 12. 13, the respirations were 28 per minute; there was still some distress, but there was no cyanosis and no signs of venous engorgement. A few râles had appeared over the left lower lobe of the lungs. The pulse-rate was 88 per minute. The blood exposed to 17 mm. oxygen pressure gave a *saturation of* 54 *per cent.* The course was towards rapid and uneventful recovery. Hollow breath sounds were heard over the left lung on 15. 1. 14, the breathing being easy. On 16. 1. 14 the patient got up; crepitations were still present but were clearing. He was discharged to a convalescent home on 22. 1. 14.

Spasmodic dyspnoea.

Lastly, we report a case of exceptional interest; a man who suffered from acute paroxysms of breathlessness of several hours duration (Case 18).

Case 18. *Paroxysmal breathlessness in a man who had signs of cardiac and renal involvement with aortic aneurysm. Exaggerated acidosis found during the attack.*

J. B., a driver of 50 years, was sent to the Out-patient Department for examination and report by Dr. Sidney Owen.

History. Of past illnesses, he gave a history of gonorrhoea at 25 years, and some 18 months before admission had suffered much from eczema and carbuncles.

Present illness. For 3 months he had suffered from breathlessness, especially upon exertion, and his work had become increasingly difficult. In the middle of June he was sitting in his car on the rank when he noticed himself more breathless than usual. In a few minutes the breathlessness increased so much as almost to suffocate him; he collapsed and became unconscious, was driven to the West London Hospital and admitted. In a few hours the attack subsided entirely, leaving him as he was before it came.

(8. 6. 14.) When he came to the Out-patient Department his condition was as follows: A strongly-built full-blooded man. with a very faint trace of cyanosis. While talking he was a little breathless, but was able to lie fully prone and at ease. The veins were a little full, the pulse regular and at normal rate. The heart was enlarged, but presented no murmurs. A few crackles were heard at both bases. Without warning, and while sitting on the couch, he began to pant, and within a few minutes he presented a picture which gave rise to anxiety for his life. The breathing was of the most laboured kind, the face quickly became cyanosed and intensely pale, sweat broke out all over him. The cyanosis was definitely of later origin than the urgent breathing. Orthopnoeic,

he literally gasped for air and had the appearance of a cardio-renal patient *in extremis.* The heart's dullness meanwhile showed no increase, but the chest had evidently enlarged and râles and rhonchi appeared universally over it. No sputum was ejected. Oxygen seemed to afford little or no relief; he passed a quantity of limpid (sp. gr. 1,008) urine (stating that he had done likewise in the former attack). An attempted venesection was almost unsuccessful at first; the veins seemed empty and seemed to refuse to fill. The pulse remained of excessive tension, the rate rising to 148 per minute. The temperature was below 96°. The blood, taken early in the venesection and exposed to oxygen, gave a *saturation of* 48 *per cent.*; eventually by deep dissection 18 ounces were drawn. The patient was semi-conscious within half an hour of the commencement of the attack, but soon began to recover. After two hours he was much more comfortable and put to bed. The pulse-rate fell to 88 within a few hours of the onset; and the respirations, which at admission were 44 per minute, fell to 24 by the same time, and distress vanished, the colour meanwhile improving conspicuously.

(9. 6. 14.) Next morning he was perfectly comfortable, having slept during the night, and the bronchitic signs having disappeared from the chest, leaving a few crackles at the bases. He was then in much the same condition as at his admission to the Out-patient Department.

(10. 6. 14.) A radiogram showed an aneurysm of the aortic arch. He lay flat in bed without distress and with slight respiratory reserve. The respirations were 28, the pulse 76 per minute. He had very slight cyanosis of the lips. The blood exposed to 17 per cent. oxygen pressure showed *saturations of* 60 *per cent. in each of two determinations.* The nervous system was found to be normal. The urine contained a cloud of albumin and was of acid reaction, sp. gr. 1,020. A blood-pressure reading taken by Dr. Owen before he was sent was 220 mm. Hg. Immediately after the venesection it was 100, but rapidly rose during the next few days to 180, and oscillated in this neighbourhood. The Wassermann reaction was negative. The temperature rose to normal as the attack subsided and remained so.

(15. 6. 14.) His condition was one of comfort. There had been no further incidents since his attack. The quantity of urine varied between 500 and 2,500 c.c. per diem, being usually as much as 1,500 or 1,600 c.c. The respirations at the time the first blood sample was taken were 20 per minute. The blood exposed to 17 mm. oxygen pressure gave an *oxygen saturation of* 65 *per cent.*

The paroxysm of breathlessness, which the report covers, and which almost killed the patient, may have been due chiefly, if not entirely, to sudden flooding of the system with acid products. Such being the case in this patient, we consider it highly desirable that attention should be directed to all forms of paroxysmal dyspnoea, from the standpoint of blood reaction during the attacks. It seems possible, if not probable, that many instances of spasmodic dyspnoea may result from a similar causation.

Degree of Acidosis in Cases 1–18.

Case.	Initials.	Date.	Percentage Saturation at 17 mm.	Acidosis in Equivalents of Lactic Acid %.
1	A. A.	8. 11. 13	66, 67	0·01
		28. 11. 13	66	0·01
2	J. L.	16. 6. 13	61	0·02
3	Mrs. G.	26. 11. 13	64–67	0·01
4	Mrs. F.	3. 12. 13	55, 56	0·03
5	T. E.	1. 10. 13	60	0·02
6	G. G.	18. 11. 13	46, 52	0·04
7	G. W.	2. 11. 13	77	0·00
8	Mrs. S.	30. 6. 13	74	0·00
9	Mrs. A.	2. 11. 13	77	0·00
10	M. B.	8. 6. 14	45 circa	0·05 circa
		15. 6. 14	53, 57	0·03
11	J. N.	24. 10. 13	43·5, 44	0·05
12	F. P.	29. 4. 14	75	0
		4. 5. 14	52, 60	0·03
		18. 5. 14	77, 79	0
13	A. L.	15. 12. 13	52, 56	0·03
14	T. D.	24. 12. 13	59, 60	0·02
		30. 12. 13	67	0·01
		26. 1. 14	70, 72	0·00 +
15	M. G.	2. 6. 14	34	0·07
16	Mrs. W.	3. 12. 13	49, 53	0·04
		10. 12. 13	66	0·01
17	W. H.	30. 12. 13	54	0·03
18	J. B.	8. 6. 14	48	0·04
		10. 6. 14	60, 60	0·02
		15. 6. 14	65	0·01

REFERENCES.

1. Lewis, Ryffel, Wolf, Cotton, and Barcroft, *Heart*, Lond., 1913-14, v. 45.
2. Lewis, *Brit. Med. Journ.*, 1913, 2. 1417.
3. Poulton and Ryffel, *Journ. of Physiol.*, Camb., 1913, xlvi. Proc. 47.
4. Straub and Schlayer, *Münch. med. Wochenschr.*, 1912, lix. i. 569.
5. Barcroft, J., *The Respiratory Function of the Blood*, 8vo, Cambridge, 1914.

IV. *The Excitatory Process in the Dog's Heart.*[*] Part II.—*The Ventricles.*

By THOMAS LEWIS *and* M. A. ROTHSCHILD.

Communicated by Prof. E. H. STARLING, *F.R.S.*

(Received December 8, 1914—Read February 11, 1915.)

(From the Cardiographic Department, University College Hospital Medical School, London.)

[PLATES 8–12.]

Historical.

In describing the results of experiments of a physiological nature it is the natural custom to discuss the observations of previous workers in the same field.

Our present subject is the excitatory process in the mammalian ventricle, and the past publications to which we shall at present refer are those of WALLER and REID[†], BAYLISS and STARLING,[‡] and lastly GOTCH.[§]

Believing, as we do, that the spread of the excitatory process in the mammalian ventricle is far more complex than any of these writers had reason to suspect, and of so special a kind as to render the methods which they adopted almost abortive, we do not feel justified in discussing these papers in any detail. With a single exception the experiments named date back many years, and antedate recent and remarkably progressive developments of cardiac physiology.

It is necessary to emphasise the fact that recent discoveries have increased our knowledge to such extent and so altered our conception of processes in the ventricle, as to place the whole question upon a different footing and to make it abundantly clear that the older methods of investigation were incapable of analysing the progress of the natural excitation wave. In this connection they become almost purely historical.

The observations of WALLER and REID, and the later observations of WALLER,[||] were undertaken upon the dying heart, either excised or beating *in situ* after decapitation of the animal. Such records as were obtained were from the heart muscle deprived of its circulation. Upon this score alone we should be compelled to rule out the conclusions of these experiments while discussing the cause of the natural excitation wave.

---

[*] The expenses of these observations have been in part defrayed by grants from the Royal Society and the Graham Research Fund. Part I appeared in these 'Transactions,' vol. 205, p. 375 (1914).

[†] 'Phil. Trans.,' vol. 178, p. 215 (1887).

[‡] 'Monthly Internat. Jour. of Anat. and Physiol.,' vol. 9, p. 256 (1892).

[§] 'Heart,' vol. 1, p. 235 (1910).

[||] 'Phil. Trans.,' vol. 180, p. 169 (1889).

[Published, June 2, 1915]

Such records as have been taken in the past have been obtained with the capillary electrometer; the curves in no case having been analysed to eliminate instrumental defects. The recent introduction of the string galvanometer has very greatly simplified our procedure, but has shown the current changes of direct leads to be far more complex than was formerly believed.

The chief question which has engaged the attention of past workers has been as to whether the base or apex of the ventricle passes first into the excitatory state. Different observers have reported different findings; WALLER concluding that the apex usually precedes the base; BAYLISS and STARLING believing "that the ventricular contraction is a single wave. starting from the base, at the auriculo-ventricular groove and spreading thence to the apex"; GOTCH believing his results to show that the contraction begins at the base, travels to the apex, and returns to the arterial base. The relative unimportance of this question, hitherto considered fundamental, will become clearer as our own results are described. The assumption, usually tacit, in all these papers is that the excitation wave travels as a simple wave propagated from muscle element to muscle element. It is this assumption which has led to a comparison of base and apex of the heart, regions not too easily defined.*

The essential question of propagation in the ventricle is, as we purpose to show, not a question of the order of base and apex, and the assumption which brings this question into prominence is, in all probability, erroneous; for the wave of contraction is not a simple and uninterrupted spread from a single region. The possible significance of the Purkinje system has been unrecognised by past workers.

Lastly, the workers in question have failed to appreciate the essential distinction between currents emanating from two sources, *intrinsic* and *extrinsic*, as we term them, according as the origin is (*a*) in excited muscle beneath the contacts, or (*b*) in excited muscle at a distance from the contacts.

As we have shown in the case of the auricle, the initial appearance of relative negativity at a given contact, which is one of a pair upon the heart, does not necessarily indicate that the muscle beneath this contact first passes into the excitatory condition; such conclusions as have been based upon this assumption are open to very serious objections. In estimating the order in which the excitation wave appears at a series of given points, it is evidently necessary that we should be able positively to identify those deflections of our curves which correspond to the arrival of the excitation beneath the individual contacts; this is not so simple a matter as might at first appear. In looking back at the records of our predecessors in this field we are confident that extrinsic effects have been read repeatedly as intrinsic effects; in other instances, it is impossible for us to recognise the all important intrinsic effects with any degree of certainty in their curves.

---

* To attempt to bring our own results into complete harmony with those of previous workers woul require extensive comparisons of electrometric and galvanometric records; a laborious task which we deem unprofitable, especially as we are unaware of the exact sites of contact used in their experiments, a very essential matter. After full consideration we are of opinion that such discordances as exist are to b explained by the different plans upon which the investigations have been carried out, and especiall to fallacious assumptions by former workers, to which we shall refer in more detail.

Since our experiments have been completed our attention has been called to the recent publication of ERFMANN.[*] This worker has used methods not dissimilar to our own; he employed two recorders and utilised one as a standard for measurement;[†] his observations are much less extensive than our own, and are often open to the objection that extrinsic and intrinsic effects are not differentiated. ERFMANN'S work will be referred to from time to time in our report.

Anatomy.

As an introduction to our paper it will be convenient briefly to describe certain anatomical observations.

The Front of the Dog's Heart.—When the dog's heart is laid bare by splitting the sternum, a large area of the right ventricle and a smaller area of the left ventricle is exposed. Of the right ventricle we see the whole conus, the base of the ventricle where it merges with the fat of the auriculo-ventricular groove, a portion of the blunt apex of this chamber, and almost the whole extent of the anterior interventricular furrow (fig. 1). The latter is marked with accuracy throughout the greater part of its course by the descending branch of the left coronary artery and its accompanying veins, and forms almost a straight line. An area of heart wall is exposed to the right (in the animal) of this line in its lower reaches, which is of considerable importance for purposes of orientation. It is bounded on the left by the descending branch of the left coronary vessel and on the right by a line convex to the right. At first this curved line may be mistaken for the interventricular junction; it is not properly so, but forms the outermost attachment of fibres of the right ventricle to the septum; it is up to this line, and not beyond it, that the right ventricle swells when distended. The enclosed area now described is relatively flat, though its surface is dimpled, for a reason which will appear subsequently. Beneath it is a sponge-like tissue, the pores of which communicate with the cavity of the right ventricle. This area is in reality a continuation of the wall of the right ventricle, but closely bound to the septum by numerous and strong trabeculæ which ascend to the septum and anterior papillary muscle of the right side; we term it the *trabeculated region* for descriptive purposes (fig. 1).

We have made numerous dissections of the whole musculature in the dog's heart and find it to be arranged on the same general plan as described for the pig and human heart by MacCallum and Mall.* We shall refer only to those features of the architecture which are essential to our thesis.

---

[*] 'Zeitschr. f. Biol.,' vol. 61, p. 155 (1913).

[†] ERFMANN employed two galvanometers side by side. His method of measurement is not stated, and errors of measurement are not estimated, but we judge it probable, both from the small amplitude of his curves and from the photographic method employed, that they were considerably greater than seems to us expedient in the estimation of small time differences.

The superficial fibres over the ventral surface of the ventricle sweep from right to left. Starting in the conus tendon and at the auriculo-ventricular ring a broad and thin sheet of fibres passes obliquely over the upper conus and crossing the interventricular groove (Plate 8, figs. 20 and 21), sweeps around the left heart margin and eventually terminates in the vortex of the left ventricle. Further to the right, fibres belonging to the same system pass to the trabeculated region, but these do not cross; they interlace in an intricate manner with fibres ascending from the left apex. The general distribution of these superficial fibres may be seen in Plate 8, figs. 20 and 21. Thus, of the superficial fibres which start at the base of the right ventricle and appear over the front, the upper ones pass to the left ventricle and sweep around it in a continuous sheet, while the lower ones are lost in the interlacement of the trabeculated region; on the surface, the change in distribution defines the upper limit of the trabeculated area. The deep distribution of the fibres of the trabeculated region may be most easily understood with the aid of a diagram (fig. 2). This diagram represents an oblique coronal section taken through this region of the heart, so arranged as to cut the large anterior papillary muscle in its length. The superficial fibres of the right ventricle are cut longitudinally and are seen at (*a*); they sweep into the trabeculated region, the limits of which are indicated by arrows, and, decussating, pass into the papillary muscle and septum through strong trabeculæ. Beneath them lies a second and thicker layer of fibres (*b*), which takes a more transverse course over the front of the heart and sweeps forward to form the main thickness of the conus proper; it enters the septum at a much higher level and takes little or no part in the formation of the trabeculæ. The fibres *a* decussate with fibres *c* which sweep over the

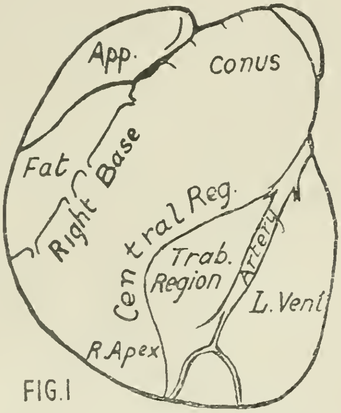


FIG. 1

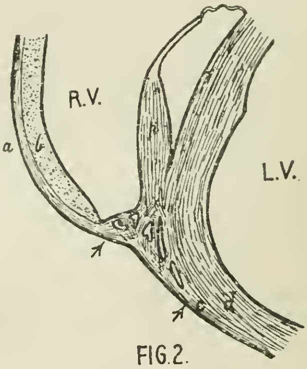


FIG. 2.

Fig. 1.—A diagram of the ventral surface of the heart of a dog.

Fig. 2.—A diagram (natural size.) illustrating the architecture of the trabeculated region, as seen in section. *p* = papillary muscle.

---

* 'Amer. Journ. Anat.,' vol. 11, p. 211 (1910–11).

left apex in one direction and form the right wall of the septum in the other. A complete and clean separation may be easily effected between the layers *c* and *d*, the latter comprising the chief fibres of the left ventricular wall. That portion of the trabeculated region which lies opposite the anterior papillary muscle may be regarded as the root of this muscle, and the dimpled surface of the heart in this region, especially prominent in the active organ, is due to this attachment of the papillary muscle. It is used as the chief landmark in estimating the position of the papillary muscle and right branch of the *A–V* bundle in the intact organ when observations upon these structures are desired.

Distribution of the Purkinje System of Fibres.—A complete and accurate account of the course of the auriculo-ventricular bundle and its two chief branches to right and left ventricles in the dog is to be found in TAWARA's important monograph.* Some idea of the distribution of the arborisation and subendocardial network of the junctional system, of which this bundle is the beginning, is to be gathered from the same publication. TAWARA's description is based upon reconstruction in serial sections, and this writer refers to the difficulty of identifying the branches of the network in his sections, on account of their close resemblance to ordinary ventricular muscle fibres. His description of the network is therefore imperfect.

The extent of the Purkinje system and the general plan of its ramifications has recently been studied in the ungulate heart by the injection of the sheaths which everywhere enclose the main strands, a method introduced by LHAMON† and since employed by a number of workers. We have also used this method extensively in the ungulate heart, for, by its means, we are able to form a very exact idea not only of the form and distribution of the chief branches, but also of the extent of the network. To certain of these observations we shall allude. In the ox heart, as has been demonstrated by TAWARA, and by LHAMON, COHN,‡ and others using the method of injection, the right division of the main bundle stem courses from the membranaceous septum, along the right side of the muscular septum to the base of the moderator band. At this point, according to COHN, branches flow to an extensive network which covers the dorsal part of the septum. The arrangement of the network is well displayed in Plate 11, fig. 24, a photograph of the opened right ventricle of an ox. The sheaths have been injected. The course of the right division of the bundle is seen as a white streak over the moderator band (*c*--*b*). This division subdivides at the base of the chief papillary muscle (*b*), but early outgoinπıy. Over are visible at *c*. That there is a definite connection in this region ɤmost continuous covering the septum of the ox, we can show clearly. sher an or denser parts of which muscular moderator band does not include the right

traverses the chamber on the conus side of it, and has a se 906); see also SCHMORL, 'Unter-

---

* 'Das Reizleitungssystem des Säugetierherzens,'

† 'Amer. Journ. of Anat.,' vol. 13, p. 55 (1912).

‡ 'Heart,' vol. 4, p. 225 (1912–13).

r outgoing fibres exist; the right
n of the papillary muscle.

An example is shown in outline in fig. 3 (*m.b.* = moderator band; *c–b*, the separate sheathed branch of the bundle). The injection fluid was allowed to flow up the sheath of this bundle (from the point marked *inj*); it quickly spread along the whole length of the right division (*d*) and, flowing out through the free branches (*a*, *a*), filled a network whose extent is indicated by the arrows on the chart. We emphasise these early outgoing branches in the ox heart because we have failed to find them in the dog. The actual network of the right ventricle in the ox is found throughout almost the whole extent of the subendocardial space (see Plate 11, fig. 24). The apical portions are everywhere lined with it, and it extends throughout the whole conus to within a

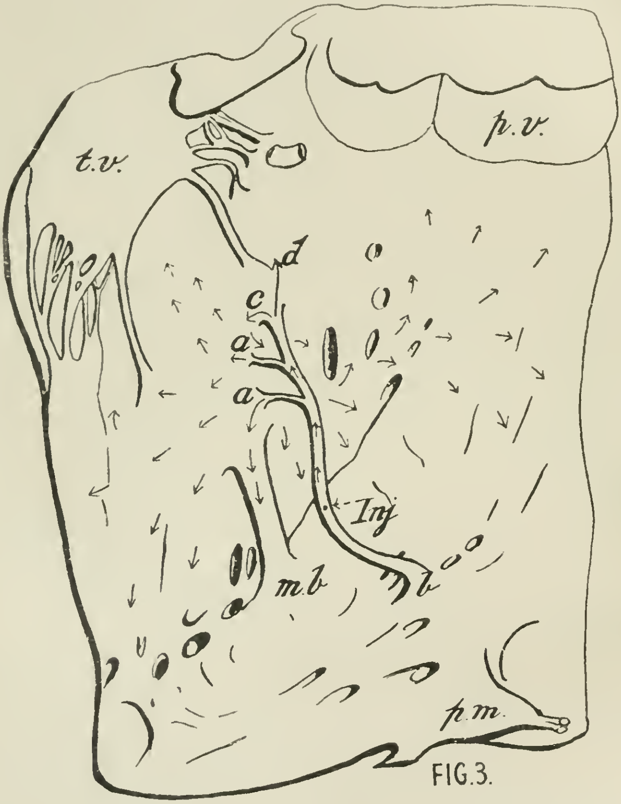


FIG. 3.

FIG. 3.—An outline drawing (⅗ natural size) of the opened right ventricle of an ox heart. *p.v.* = pulmonary valves; *t.v.* = septal segment of tricuspid valve; *p m.* = papillary muscle; *m.b.* = moderator band; *c–b* = right division of bundle specially ensheathed with endocardium. Colouring matter was injected upwards at the point marked *inj.*; it flowed in the sheaths of the Purkinje tissue over the areas ted by arrows.

tudinally
of which are the bases of the pulmonary valves. It extends towards the
and septum through ong septal wall of the ventricle, but on the latter not
layer of fibres (*b*), which ta shoots extend so far as to be concealed by the septal
heart and sweeps forward ubendocardial space deep to this valve segment is poorly
enters the septum at a muc
tion of the trabeculæ. T ection method has been found to be impracticable, though
* ‘A ently. We have used another method of displaying the

system. We have taken advantage of BEST's* method of glycogen staining, knowing from NAGAYO's studies† that the glycogen content of the special tissues is high. At the end of an experiment the animal is kept alive, and 150–300 c.c. of a saturated solution of dextrose is slowly run into the bloodstream. After one or two hours the dog is killed, and the heart quickly removed and washed in a saturated dextrose solution. It is distended with 35 per cent. formalin solution also saturated with sugar and left until hardened. After being again washed in saturated dextrose solution it is stained in bulk with Best's alkaline carmine for from 12 hours to a week, and differentiated in his alcoholic fluid. After 12 or more hours the whole Purkinje network and the chief branches of the bundle are well displayed *in situ* as dark red lines upon a pink background.

In the dog, the right bundle division passes deep to the conus edge of the septal segment of the tricuspid valve (Plate 8, fig. 19), and is almost always superficial in its course from this point onwards; it follows a curved course over the septum, reaching and embracing the base of the chief papillary muscle. Arriving at the right and dorsal border of this muscle it breaks up into a number of branches, and many of these then proceed across the cavity of the ventricle to the free wall, each isolated and sheathed by endocardium (Plates 8 and 10, figs. 20, 21, 23). Frequently a complex interlacement extends dorsally from these free branches, and twigs are sent to the apical portions of the endocardium in many directions. Reaching the wall of the ventricle, the branches and twigs break up and are lost in the network proper. In our experience every free strand of considerable size in this region is a carrier of Purkinje substance. But these are not the first branches of the right division. The earliest outgoing fibres which we can discover come from the convexity of the right division, as it sweeps around the papillary muscle (see diagrams below, figs. 12 and 13, and Plate 8, fig. 19). These course along the trabeculæ from septum to free wall. We are unable to find early outgoing fibres to the septum towards the base of the heart, either in studying macroscopic specimens, or in the histological preparations similarly stained.‡ In a solitary heart a single branch of early origin was discovered, but this ran, seemingly, as an unbranching strand, almost to the apex of the ventricle along the septum dorsally to the chief papillary muscle. The network has a very similar distribution to that found in the ox, though our method displays it more fully than it can be seen in the last-named animal. It may be said to line the subendocardial space universally with the exception of areas to be mentioned presently. Over the free wall of the right ventricle, including the conus, it forms an almost continuous sheet; like dense lace it forms an almost imperforate sheet, the denser parts of which

---

[*] BEST, 'Zeitschr. f. wissensch. Mikroskopie,' vol. 23, p. 319 (1906); see also SCHMORL, 'Untersuchungen Methoden,' 7th Ed., p. 183.

[†] 'Verhandl. d. deutsch. pathol. Gesell.,' vol. 12, p. 150 (1908).

[‡] Experimental evidence has since been obtained, however, that earlier outgoing fibres exist; the right division of the bundle does not pass entire and unbranched to the region of the papillary muscle.

cover over the raised trabeculæ, the fibres in the sheet being arranged side by side and following faithfully the directions of the smallest trabeculæ Thus every depression is encircled by a broad band of parallel fibres. The depressions themselves are lined more sparsely, but the whole subendocardial space is supplied. If strips are torn from the endocardium and examined under a microscope, a finer network is seen filling the interstices in the coarser network. Where, as in the upper reaches of the conus, depressions are less defined or absent, the same general arrangement holds good ; broad bands of the tissue are looped together and encircle spaces in which a flimsier network is discovered. It seems as though this region were also once trabeculated, but that with the expansion of the conus the irregularities of the surface have been lost. The special tissue runs into the deep sulci of the freely trabeculated space along the ventral attachment of the right ventricular wall (the trabecular region already described). The septal subendocardial region is less richly supplied ; the network does not appear to be well developed towards the tricuspid valve, once the papillary muscle is passed. The base of this muscle is richly supplied, but above it, strands visible to the naked eye are rarely seen, and under the microscope only occasional traces of a flimsy network are discovered. No fibres have been seen deep to the septal valve segment itself. There appears, therefore, to be an area of subendocardial tissue in the region of this valve segment, poorly supplied by the network or actually devoid of it. On the free wall and towards the tricuspid valve the extension is higher, though it falls short of the auriculo-ventricular ring itself.

The general arrangement in the left heart is similar to the recognised distribution in ungulate hearts. The left division breaks into two chief strands, and these proceed, completely enwrapped by endocardium, to basketworks around the anterior and posterior muscles (Plate 9, fig. 22). Other and smaller branches run down the septum branching and anastomosing freely. The network over the whole apical half of the ventricle is extremely dense and similar in its general constitution to that found in the other ventricle. A sheet of interlacing strands passes deep to the unattached portion of each papillary muscle ; the whole subendocardial space is richly lined except for a small area immediately beneath the aortic valves on each side of the main left division.

It is most important for our purposes that the general and wide distribution of the Purkinje system should be grasped, and that certain special features should be remembered, for these features serve to explain some of the experimental facts presently to be detailed. In particular we would emphasise the course and relation of the right division to the chief papillary muscle of the right ventricle (see Plates 8–10, figs. 21, 22, and 23); the presence of a complex system of large free or bridging strands in the right chamber ; the fan-shaped distribution in the left chamber ; and lastly, the presence of two areas which are comparatively free from fibres, the first and largest being on the right side of the septum in the region of the septal segment of the tricuspid valve, the second immediately beneath the aortic valves. It is also of importance to point out that in the dog, as in the ox, the distribution is not confined to the sub-endocardial space; for sections clearly demonstrate that here and there offshoots pass into the muscle substance, following the course of vessels or connective tissue strands. Such penetration is not deep so far as we can ascertain; it is of one or more millimetres extent; neither is it frequent; but where the wall of the ventricle is thin, it may bring the special tissue very near to the pericardial surface.

Experimental Method.

Our methods are described in detail in the section of our reports which deals with the auricle, consequently it is necessary to record only those variations of technique which have been necessitated by the transference of observation from auricle to ventricle. In all experiments the vagi have been divided. We expose the heart in one of three ways: by splitting the sternum, the animal lying on its back; by removing a large area of the right chest wall, the animal lying on its left side; or, finally, by removing a large area of the left chest wall, the animal lying on its right side. We adopt one or other procedure in a given experiment, maintaining the attitude throughout that experiment. The several exposures permit us to examine the whole superficies of the ventricle.

The electrodes for direct leads which we have been employing have been of three classes:—

1. Paired contacts such as were used for the auricle.

2. Solitary contacts of the same pattern, though smaller in bore (*i.e.* $1\frac{1}{2}$ mm.), and used in conjunction with non-polarisable contacts stitched permanently to right or left chest wall.

3. All-glass electrodes, with suitable curvatures for observation inside the cavities of the ventricles. At first these electrodes were double; eventually, and for the majority of the observations, we have employed single contacts, and have led from these to a contact on the chest wall.

Our standard signal has been Lead *II*, from right shoulder to left groin, and the sensitivity of the recorder for this lead has been such that 3 millivolts introduced give an excursion of 3 cm. For direct leads the sensitivity has varied according to circumstances; but as a general rule it has been such that 6 millivolts give 3 mm. (scale divisions in our figures) excursion.

The experiments have been so arranged that all the observations upon a given animal have been made with as little displacement of the heart as possible. For external leads there is no displacement; for internal leads there is little; the latter have been undertaken for the most part by introducing contacts through one or other auricular appendix and the corresponding auriculo-ventricular orifice. Internal leads have always been the last in an experiment.

At the time of the experiment careful drawings, so far as possible to scale, have been made of the exposed heart, a number of the vessels being charted to aid the subsequent identification of contact points. In some instances it has been necessary to mark the epicardium at the time by staining it with a small spot of carmine. The heart has been excised at the end of the experiment, distended, and fixed. Subsequently the contact points are identified and an accurate drawing to scale has been made either by measurement or by optical projection. Certain of the hearts were prepared in the special manner already described to display the Purkinje network.

Our standard, in estimating with the comparator the time at which the excitation wave appears at a given point, is *R* in Lead *II*; this deflection starts sharply, and as a rule requires no check. Its beginning is subject to far less error in estimation than is *P*, the auricular summit, adopted in our previous observations.

Recognition of Intrinsic Deflections.

It will be remembered that our observations upon the auricle depended upon our ability to isolate and recognise that deflection of our galvanometric fibre which, in records from direct leads, corresponds to the arrival of the excitation process beneath one of the contacts placed upon the muscle. In a direct lead the first deflection of the string, as we were able to show, does not usually correspond to the passage of the muscle beneath the contacts into the excitatory state. We divided the deflections obtained in direct leads into two classes for purposes of description, terming them intrinsic and extrinsic respectively. In the case of the auricle, to recognise the intrinsic deflection, or that which signals the arrival of the excitation at the contact area, is not difficult; for the spread of the excitation wave takes place in a radial fashion from the sino-auricular node, and so if the paired contacts are arranged radially to this node, and the same contact is maintained as the proximal contact throughout, the intrinsic deflections are all in the same direction upon our plates. Further, the time of a given intrinsic deflection may be taken as the time at which the excitation process arrives at the corresponding proximal contact. When we came to study the ventricle we were met by an initial difficulty. We had no preliminary guide to the direction taken by the excitation wave; so, when we placed a pair of contacts upon the ventricle and obtained a complex curve consisting of a number of upward and downward deflections, we might be unable to state from its appearance which contact had first received the excitation wave; it not infrequently happened that we were unable to identify our intrinsic deflection with any degree of certainty. We performed a large number of experiments in which various areas on the surface of the ventricle were examined by means of paired contacts and, although these experiments gave us a general idea of the events, the method did not provide us with data of sufficient accuracy; and, ultimately, we were obliged to discard it. If closely paired contacts are placed upon the surface of the ventricle and connected to the galvanometer, the direction of the intrinsic deflection is usually obvious, for this deflection is the only one which is of considerable amplitude as a general rule. In our first studies we were able to map out the general order of the muscular activity by watching the moving fibre, and by using the direction of the chief deflection as a guide to the initial negativity of one contact as compared to the other. If this method is used for the front of the heart it is found that the earliest region to show the excitatory state is a point or band on the ventral surface of the right ventricle where its wall becomes attached to the septum and near the middle of this line of junction: that is to say, where the free wall of the ventricle borders the middle of what we term the trabeculated region. For descriptive purposes we name this region, which shows the first sign of passing into the excitatory state, the *central region* (fig. 1). If the plane of the contacts is maintained across the heart, and the contacts are moved over the ventral surface of the right ventricle (speaking of the free wall), a contact on the left border of this ventricle will always show relative negativity to a contact placed nearer its right margin, indicating that the general course of the excitation wave is from left to right, so far as this ventricle is concerned. If we examine the same ventricle with the contacts on lines following the long axis of the heart, then the general course of the excitation wave is towards the base over the basal two-thirds* and towards the apex over the apical third of the right ventricle. Measurement of the times at which the intrinsic deflections appear at the different contact points relative to *R* in our standard lead gave us a general and clear confirmation of these results, and we were satisfied that the earliest region to become excited is at the left border of the right ventricle. But when we attempted to map out the heart in detail by this method of paired contacts, and especially when we came to examine the left ventricle, we were unable to proceed with the certainty which we desired. For it often happened that the deflections from a pair of contacts were extremely numerous, and two or more might have similar amplitudes; consequently, we could not finally decide upon a given deflection as the intrinsic deflection. Curves of this kind, as we have been able to show subsequently, are due to the two contacts resting on points which enter the excitatory state almost simultaneously. This condition is especially frequent over the left ventricle.

For this reason we adopted another method of leading off, using a single contact upon the muscle and maintaining a second contact upon some fixed point on the chest wall. This method has the advantage that all intrinsic deflections have a constant direction (upwards in our curves); as a consequence they can be recognised with greater facility and certainty. Where, as in Plate 11, fig. 26 B (upper curve), the curve obtained has consisted of a minute or small downward deflection followed by a prominent upward spike, and where this spike is the most abrupt (as judged by the thinness of the written line) and has by far the greatest amplitude of any deflection in the curve, we are confident it represents the intrinsic deflection and corresponds to the arrival of the excitation process beneath our solitary muscle contact. Where the curve is of less distinctive form, its form may be altered oftentimes by moving the distal

---

* ERFMANN, using a different method of leading, comes to a precisely opposite conclusion.

(or chest wall) contact either to another point on the chest wall or on to the heart itself. Moving the distal contact has been employed to confirm our interpretation of the events represented in a curve, where such confirmation has seemed desirable. We find in the ventricle precisely what we found in the auricle, namely, that a change in the position of the distal contact* does not materially influence our measurements; yet such change may modify the shape of the curve profoundly. A number of measurements which bear out this statement are given in Table I. The last observation in this Table is illustrated by Plate 11, fig. 25 A and B. The curve obtained from contacts *a–b* (fig. 4) is shown in fig. 25 A. It consists of a number of small deflections, succeeded by a prominent summit, which has its onset 0·0188 second after *R*. The curve from contacts *a–c* (fig. 4), *c* being upon the left chest wall, is of an entirely different type (fig. 25 B). In this figure the prominent and steep upward movement represents the intrinsic deflection, though it starts well below the base line; it comes at almost exactly the same time interval, namely, 0·0190 second after *R*. Each curve gives a sufficiently accurate reading, but the time at which the excitation wave arrives at *a* is known with greater certainty from the second curve (fig. 25 B), for in the case of this curve

TABLE I.—Changing Distal Contact.
(Extrinsic and Intrinsic Deflections related to *R* in Lead *II*.)

Dog.	Proximal contact.	Distal contact.	Extrinsic.	Intrinsic.
G.F.	1. Central region	1 cm. above proximal	–0·0078	0·0055
	2. Unchanged	Right chest wall	–0·0099	0·0037
G.H.	1. Central region	1 cm. away from proximal	–0·0070	0·0042
	2. Unchanged	Right chest wall	–0·0081	0·0035
G.H.	1. On front of L.V.	Right chest wall	–0·0071	0·0130
	2. Unchanged	Left chest wall	–0·0079	0·0132
	3. Unchanged	Mid abdominal wall	–0·0073	0·0124
G.H.	1. Mid R.V.	1 cm. above proximal	–0·0031	0·0027
	2. Unchanged	Conus	–0·0056	0·0036
G.I.	1. Apex of R.V.	Base of R.V.	–0·0089	0·0096
	2. Unchanged	Chest wall	–0·0094	0·0096
G.J.	1. Base of R.V.	8 mm. above proximal	–0·0112	0·0014
	2. Unchanged	Right chest wall	–0·0121	0·0009
G.J.	1. Mid front L.V.	Left chest wall	–0·0100	0·0163
	2. Unchanged	Right chest wall	–0·0097	0·0164
G.J.	1. Mid front L.V.	8 mm. above proximal	–0·0066	0·0188
	2. Unchanged	Left chest wall	–0·0100	0·0190

NOTE.—All readings in our Tables are averages for three heart cycles.

---

* Meaning at the present time by distal contact, that contact which is the last to receive the excitation wave, as opposed to the proximal contact which receives it first.

we know from the arrangement of the contacts that an upward movement signals relative negativity of the single contact upon the heart.

It must be evident that it is a matter of first concern to us, to ensure a correct interpretation of our curves. The procedure just described, namely, movement of the distal contact, is our first method of control. A second and equally satisfactory control is also similar to a method adopted for the auricle. We found in studying this chamber of the heart, that the intrinsic and extrinsic deflections could be separated and identified by taking a series of curves from serially arranged contacts over the edge of the auricular muscle on inferior and superior cava. A similar procedure is possible in the case of the pulmonary artery. A series of readings is taken from contacts lying on the conus and pulmonary artery; an example of such readings is given in fig. 5.

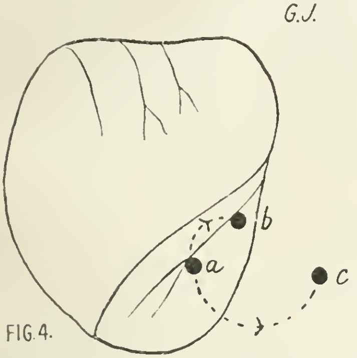


FIG. 4.

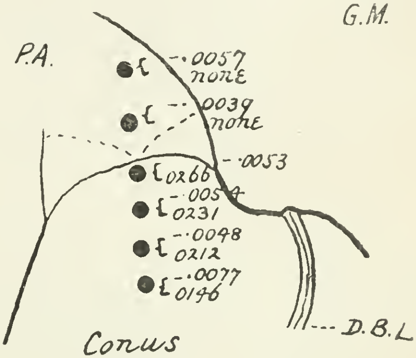


FIG. 5.

Fig. 4.—A diagram to illustrate a comparison between two methods of leading from the heart. 1. Two contacts, *a* and *b*, are placed upon the muscle, and the lead is from *a–b*. 2. A contact, *c*, is placed on the chest wall, and the lead is from *a–c*. The contact from which an arrow points is that which when relatively negative yields an upright spike in the curve. The corresponding curves are shown in Plate 11, fig. 25.

Fig. 5.—An outline (natural size) of the conus and pulmonary artery (P.A.), showing readings of extrinsic deflections (above) and intrinsic inflections (below) from six contacts in a line over conus and artery. D.B.L. = descending branch of left coronary artery.

Opposite each contact site are two figures; the upper one is the time, relative to *R*, at which the first extrinsic deflection appears; the lower one is the time of the corresponding intrinsic deflection. The extrinsic deflections of this series appear at times which are within a few thousandths of a second of each other, and in no definite order. The times of the intrinsic deflections are arranged in an orderly fashion, becoming later as we proceed up the conus; but as soon as the muscle edge is passed, the intrinsic deflection vanishes, showing that, when present, it is dependent upon the activity of muscle immediately beneath the corresponding contact. A similar series of conus leads is shown in fig. 11.

The Form and Amplitudes of Ventricular Curves.

The form of curves taken directly from the ventricle by a single contact is variable. The majority of such curves show an initial dip of variable extent (2–35 millivolts).

This dip is relatively slow and usually unbroken, but it may be notched; it represents the first or extrinsic deflection. There may be a return to the base line before the intrinsic deflection is recorded, but more commonly, as in Plate 11, fig. 25 B, the onset of the intrinsic deflection terminates the dip. The upstroke is always very abrupt, and the recorded image of the fibre is consequently faint. Its amplitude, measured from its point of origin, is almost always considerable (10–80 millivolts). Usually, therefore, the intrinsic deflection is recognised with facility. In exceptional curves and where it is preceded by deep extrinsic effects of long duration, it may not be so easy to identify; in some such curves it consists of a short spike interrupting the downstroke. In other instances, its onset may be confused by the presence of several preliminary extrinsic deflections; if immediately preceded, as it may be, by an upward movement of small amplitude, this upstroke and the intrinsic deflection itself may fuse and an inaccuracy is introduced into the readings. But readings from such curves can almost always be avoided, and the onset of the deflection which represents the curves of the excitation wave can be identified positively in the great majority of the curves.

Distribution of the Excitation Wave over the Surface.

Ventral Surface.

We have studied the distribution of the excitatory process on the surface of the ventricles in more than 30 animals, and, using the methods which have been described, have arrived at results of considerable constancy. It is not possible satisfactorily to examine the whole surface in a single animal, and we first studied the ventral surface, as it is exposed by splitting the sternum. Our observations consisted in ascertaining the times at which the excitation wave appeared, relative to *R* in Lead *II*, at some 20 or more points, freely utilising the surface vessels and other landmarks for accurate charting. In Plates 8 and 10, figs. 20, 21, and 23, three projected outline drawings are published which sufficiently illustrate our results. Speaking of this, the ventral surface of the heart, the first appearance of the excitation wave is almost simultaneous with *R* in Lead *II*. Five points in fig. 20 show readings (0·0037, 0·0051, 0·0055, 0·0067, and 0·0073 second) varying no more than 0·004 second from each other. These points lie in our central region and are surrounded by points showing readings varying from 0·0108 to 0·0131 or 0·0145. All points over the right ventricle are later than those of the central region, and they are later according as they are further removed from it. Thus the excitation wave appears at the base of the right ventricle at times such as 0·0267 and 0·0221 second. If we examine the similar diagrams of figs. 21 and 23, we see a similarity of distribution. The central region (right ventricle) comes first in order, but the earliest region is as a rule not confined but diffuse. The limits and extent of this central region vary a good deal from animal to animal, but the present examples sufficiently illustrate them. In practically all our animals the base of the right ventricle, where it runs into the fat of the groove, comes between 0·0150 and 0·0270 second (the last value is exceptionally high). The conus is later, and near its exit nearly always surpasses 0·02 second and approaches 0·03 second. The trabeculated region, included between the bulge of the right ventricle and the descending coronary artery, is early (Plate 8, fig. 21, 0·0049, 0·0082, and 0·0100 second) but is later than the central region. Once the descending coronary is crossed, higher values are found; immediately to the left of it they are in the neighbourhood of 0·02 second. further to the left and along the actual left border of the heart they fall again.*

The three diagrams of the ventral surface of the ventricle are accompanied by accurate projections of the underlying structures; the relation of the surface points examined to the superficial muscle bands, the cavities, the papillary muscle, and the free branches of the conducting system are shown.

If those contacts are studied which lie over the long band of muscle fibres, springing from the base of the right ventricle and sweeping to the left ventricle across the interventricular furrow (left marginal figure of fig. 21), such contacts show almost simultaneous reception of the excitation wave. A superficial examination of figs. 20 and 21 immediately shows numerous discrepancies between the sequence of contact points and the line of the fibre; the distribution of the excitation wave is not related to the sweep of the muscle bands.

The same figures, however, give us a distinct hint as to the manner in which the wave is distributed, for there is an unmistakable relation of the earliest points to the spread of the chief branches of the right division of the bundle. The central region is the region of the free wall which is supplied by the earliest outgoing branches, namely, those which spring from the convexity of the right division as it embraces the papillary muscle, and by the free branches which bridge the cavity; the main division and these free branches are clearly depicted in our diagrams. Before we consider this question of the course of the wave more fully, we may complete our account of the surface distribution.

Right Surface.

Having obtained a complete picture of the ventral surface, and observing that the figures for any given region are relatively constant from animal to animal, we may build up the picture and include other heart surfaces by overlapping the fields of observation. This method avoids displacement of the heart in any given animal during the course of an experiment, for only a single surface is examined in a given heart. Three figures (figs. 6, 7, and 8) of the right surface are given as illustrations. The fields of observation include the whole of the right and dorsal portions of right ventricle, and a complete base-apex strip of left ventricle. The whole or part of the central region is also included. In each diagram the central region is the earliest part of the right ventricle to be excited. It is also to be noticed that the apex of the right ventricle may be included amongst the earlier regions (figs. 6 and 7); on the other hand, it may be relatively late (fig. 8). Following the descending branch of the right coronary artery, the times decrease. The same tendency is seen in moving from the base (dorsally) of the left ventricle to its apex. The figure for the actual apex of the left ventricle, *i.e.* the vortex, is always low (figs. 7, 8, 9, and 10), and often this region rivals the central region on the ventral surface of the right ventricle, in the race for priority.

---

* According to EBFMANN all points of the superficies are activated simultaneously, a few thousandths of a second only being found between them. We think this conclusion is due to the fact that his observations were almost confined to the base and left apex of the ventral surface.

Left Surface.

The left surface includes a portion of the conus, the whole of the left surface of the left ventricle, and the greater part of its ventral and dorsal surfaces (figs. 9, 10, and 11).

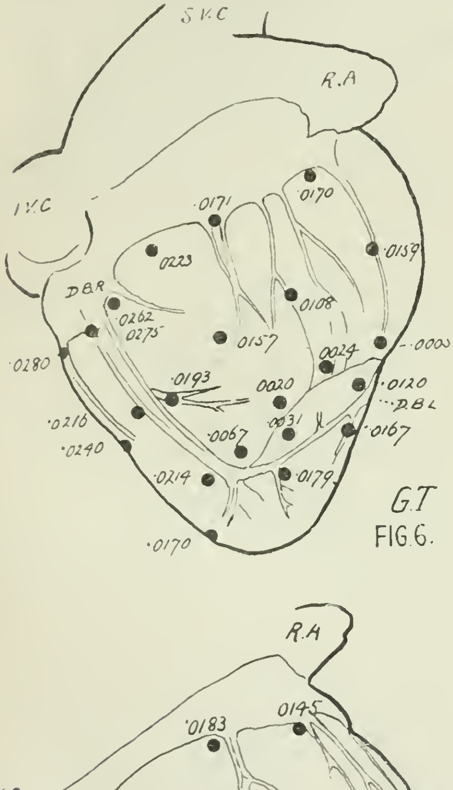


FIG.6.

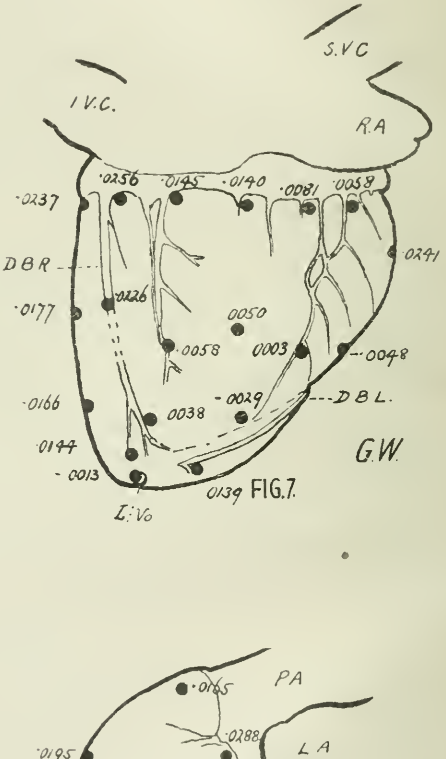


FIG.7.

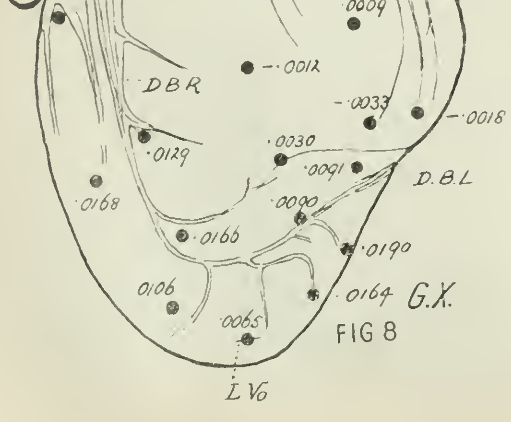


FIG 8

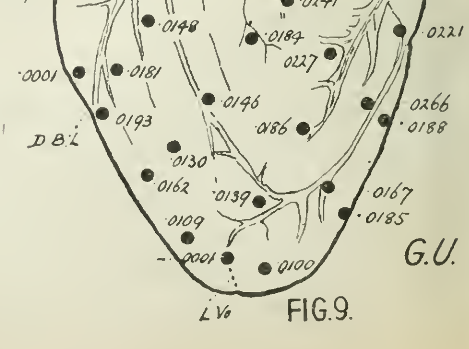


FIG.9.

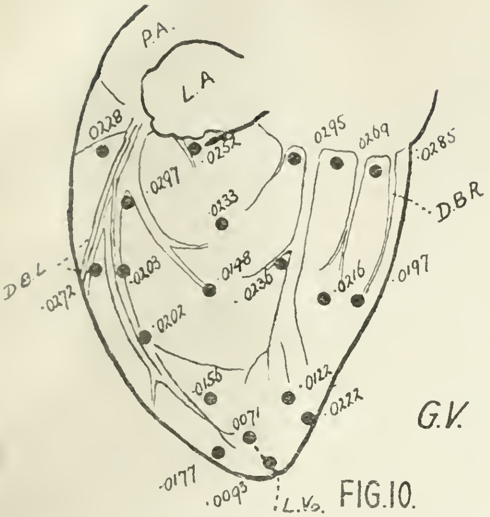


FIG. 10.

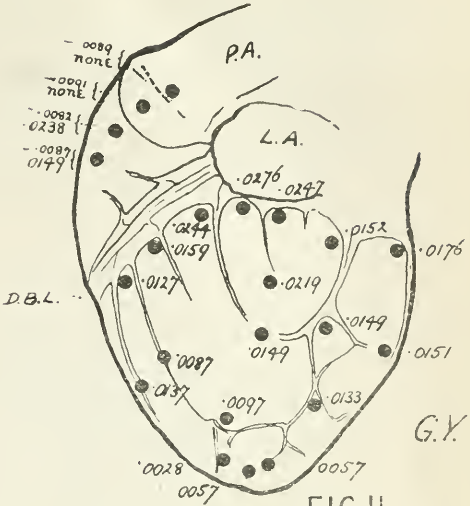


FIG. 11

FIGS. 6, 7, and 8.—Outlines ($\frac{3}{4}$ natural size) to scale of the right surface of three dogs' hearts (G.T., G.W., and G.X.); giving the time-readings of the intrinsic deflections at a number of points. The view of the heart is not quite the same in the three diagrams. S.V.C. = superior vena cava; I.V.C. = inferior vena cava; R.A. = right appendix; D.B.R. = descending branch of right coronary artery; D.B.L. = descending branch of left artery; L.Vo. = vortex of left ventricle.

FIGS. 9, 10, and 11.—Outlines ($\frac{3}{4}$ natural size) to scale of the left surface of three dogs' hearts (G.U., G.V., and G.Y.); giving time-readings at a number of contacts. L.A. = left appendix; P.A. = pulmonary artery; D.B.R. = descending branch of right coronary artery; D.B.L. = descending branch of left artery; L.Vo. = vortex of left ventricle.

The base of the left ventricle is relatively late, the figures decrease, though not with absolute uniformity, as the apex is approached. A row of contacts near and parallel to the descending branch of the left coronary is always later than the row lying immediately to the left of it. It would be more correct, perhaps, to say that a number of points on the left ventricular surface are excited almost simultaneously, that the base lags a little, as does also the ventral border, while, when the apex is approached, there is a fall in the values; a fall which in many hearts is abrupt and limited to a relatively small area.

The Surface as a Whole.

The distribution of the excitation wave over the surface of the ventricle teaches us much in regard to the distribution through the ventricle as a whole. That the distribution does not follow the superficial muscle paths has been indicated already. That the spread is not a simple spread from muscle element to muscle element is strongly suggested by further considerations; for, both upon the surface of the right ventricle and upon the surface of the left ventricle, there are numbers of points at which the excitation wave arrives simultaneously. Furthermore, the wave appears almost simultaneously over certain parts of the right ventricle, and over certain parts of the left, areas far removed from each other. Thus the central region of the right ventricle and the vortex of the left ventricle are frequently activated at almost the same moment; again, there is never a very material time interval between the excitation of the right and left bases at opposite margins of the heart. An assumption that the ventricle is first excited in a single region and that the active state is propagated in an orderly succession from this region and over both chambers, or over an entire ventricle, is incompatible with these observations. At the same time, the distribution is ordered upon a definite plan from which there is little departure from heart to heart. It is for this reason that the axial electrocardiogram is relatively constant in form. We are forced to assume, in the case of each heart examined, that propagation to the surface is along a large number of distinct channels. The nature of these channels is especially suggested by the relation of the earliest points of right ventricular activity to the underlying branches of the Purkinje system. The surface distribution over the greater part of the left ventricle is also compatible with the fan-like arrangement of the left Purkinje system. The division of the main stem of the bundle into right and left chief branches explains the earliest appearance of the excitation wave almost simultaneously over right and left ventricle. Compared to the distribution over the surface of the auricle, there are several remarkable contrasts. The surface of the entire ventricle becomes active in two or three hundredths of a second; in the auricle of dogs of the same weight, five hundredths of a second or more elapse before the spread is complete over chambers of much smaller dimensions; yet, as we shall presently show, the rate of propagation in the muscle of the ventricle is actually much slower than in the muscle of the auricle. In brief, propagation through ventricular muscular elements alone cannot account for the observed surface distribution. In the light of these observations it becomes clear that the much discussed questions as to whether right or left ventricle first becomes active, or as to which ventricle first contracts, are of minor importance. Neither chamber can be stated to be the first to develop surface activity. Areas of the right precede areas of the left, areas of the left precede areas of the right. The most important general feature is the rapidity with which all regions are activated.

It will also be clear that the question of priority of base or apex is also of little consequence. It is true that the apex of the right ventricle is usually active before any basal point on the surface of the same chamber, but it may pass simultaneously with certain regions of the base into activity, or may on occasion actually follow them. In any case, the interval is but a hundredth of a second, or a little more or less. Moreover, neither base nor apex is first; the first point is always situate in the central region.

In the case of the left ventricle, the vortex always leads, and if this point may be taken as the apex, then the apex of this ventricle is always active before the rest of the ventricle; but the surface no more than a centimetre away may be a hundredth part of a second later, or more, and may be as late as other points far removed from it.

The Direct Proof of a Purkinje as Opposed to a Muscle Distribution.

Before our report is complete we shall advance reasons for the view that the surface distribution is not governed by the Purkinje arrangement alone; another factor plays an important part. But at the present it may be well to describe certain experiments which clearly establish the importance of the Purkinje system.

Section of the Right Bundle Division.

The change in an axial electrocardiogram which follows section of the main right branch of the bundle has been described by EPPINGER and ROTHBERGER.* It is very profound. The change in the curve might, in itself, be taken as proof of change in the sequence of muscular activity. We have thought it desirable to obtain clear evidence that this is so. We cut the right branch by introducing a specially contrived and guarded knife into the ventricle through the right auricular appendix. The instrument ends in a blunt point which is passed through the tricuspid orifice and thence to the septal side of the large papillary muscle; it is felt through the thin wall of the right ventricle where this joins the septum and immediately above the dimple in the trabeculated area, which forms the root of the papillary muscle. Using these landmarks, the right branch may be divided with practical certainty and cleanly, just before it curves around the papillary muscle. That the division has been complete is ascertained subsequently, the heart being specially prepared and stained for this purpose. Two examples are shown in figs. 12 and 13; the corresponding scale drawings of the incisions are placed beneath the main figures. In the heart of fig. 12 two rows of points were chosen, having special regard to their relation to vessels for the subsequent replacement of contacts. The times at which the excitation wave appeared at the nine contacts are written on the diagram; the top figure of each pair representing the reading before, the bottom figure of each pair the reading after, the section. We may first examine the effect upon the left ventricle. Four contacts lie on the left ventricle, and the section of the right bundle division is without effect. The order in which these points become active is precisely what it was. The reading before section is in each instance later than the reading after section by 1–2 thousandths of a second. This change is attributable to a change in the standard of measurement; for, after the section, *R* our original standard is lost and is replaced by the opening phases of an electrocardiogram of different but characteristic type (see Plate 11, fig. 26 C and D). Of the remaining contacts, four lie on the free wall and the fifth over the trabeculated region of the right ventricle. It is these five contacts which show conspicuous change. At all of them the excitation wave is markedly delayed and along each line it progresses from left to right. Whereas the contact over the edge of the trabeculated region was the first to show activity originally, after section, it became later than any contact over the left ventricle.

---

* 'Zeitschr. f. Klin. Med.,' vol. 70, p. 1 (1910).

Similar changes are to be observed in the diagram of the second experiment (fig. 13); four contacts on the left ventricle show practically no change other than what may be accounted for by the alteration of standard, errors of measurement, and replacement of contacts; a conspicuous delay is seen at the two contacts on the trabeculated region and at the four contacts on the free wall of the right ventricle. Originally, the first contact to receive the excitation wave lay on the free wall opposite the centre of the trabeculated region (0·0027); after section the order changes and, as in fig. 12, there is a uniform sequence from left to rig nt over the right ventricle. After the section the wave of the right ventricle wa evidently transmitted from the left ventricle to the right in both experiments. Plate 11, figs. 26 and 27, are examples of the simultaneous curves from which the

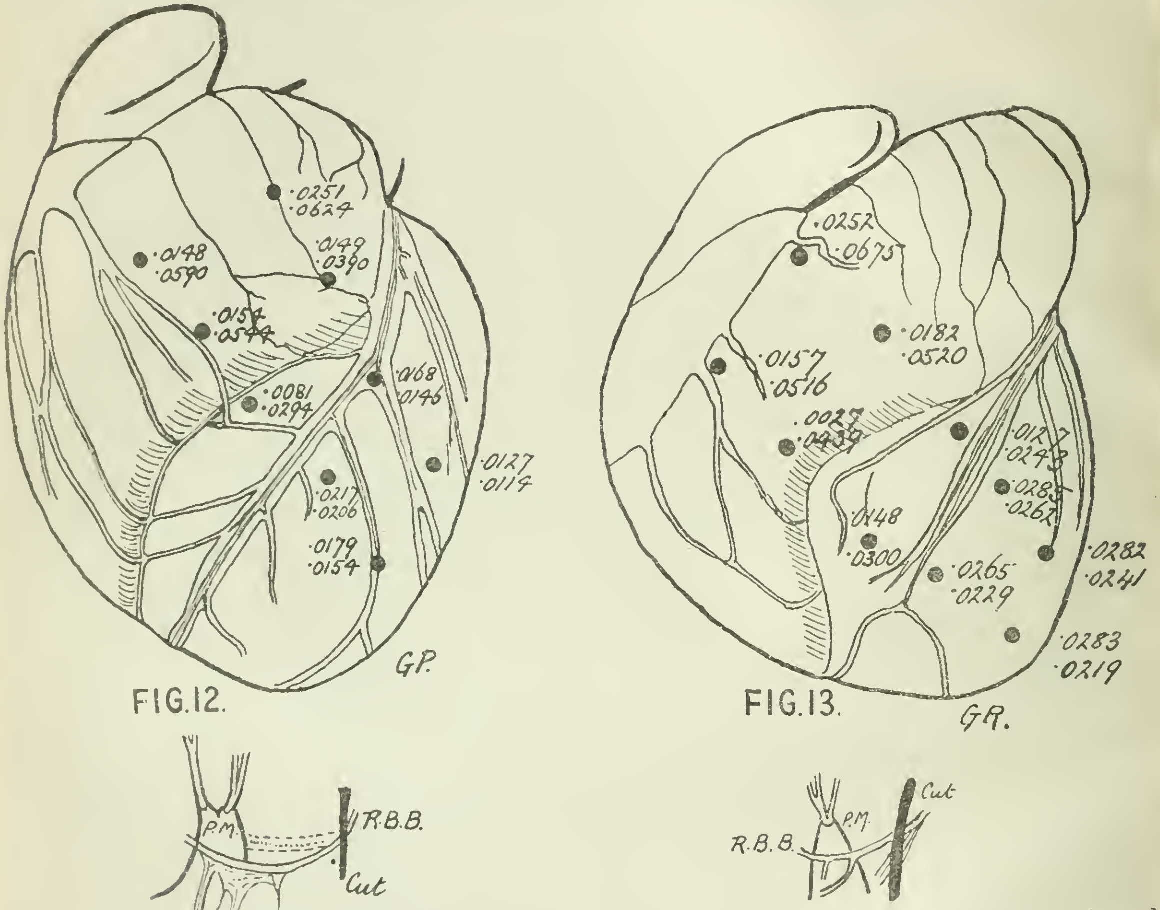


FIG. 12.

FIG. 13.

FIGS. 12 and 13.—Outlines (natural size) to scale of the ventral surfaces of two dogs' hearts (G.P. and G.R.); showing readings at series of contacts before (top figure) and after (bottom figure) transection of the right bundle division. The relation of the cut to the bundle division and the papillary muscle in the corresponding heart is shown below in scale drawings. P.M. = papillary muscle; R.B.B. = right branch of bundle.

readings on our diagrams have been obtained. In fig. 26 A and B, direct curves from two of the contacts are shown simultaneously with the normal standard curve from Lead *II*; in fig. 26 C and D, direct curves from the same two points are shown simultaneously with the altered electrocardiogram, the result of section. Plate 11, fig. 27, is similarly arranged. The measured interval *R* or *R'* to corresponding intrinsic deflection (*In*) is written on each curve.

Endocardial Damage.

If a contact is placed upon the conus arteriosus and the time at which the excitation wave appears is estimated, and is re-estimated after a knife edge has been drawn across the base of the conus internally, so that the endocardium is cut or scratched, a change is noted. There is a delay in the appearance of the excitation wave after section. Four experiments of this kind are tabulated in Table II. Here the time difference between two contacts, one below and the other above the section, is given before and after the section. (A diagram of the corresponding pericardial cut is given in fig. 14.)

TABLE II.—Natural Conus Intrinsic Deflection before and after Endocardial Section. (Readings related to intrinsic from point below section.)

Dog.	Length of incision.	Depth.	Thickness of wall.	Intrinsic.	
				Before.	After.
H.D.	30 mm.	$1\frac{1}{2}$–4 mm.	4–5 mm.	−0·0015	0·0028
H.E.	27 ,,	0–3 ,,	5–6 ,,	0·0100	0·0330
H.F.	Endocardium scratched in places over a line of 15 mm.		2–5 ,,	0·0120	0·0334
H.G.	Endocardium scratched over 20 mm.		5 ,,	0·0031	0·0238

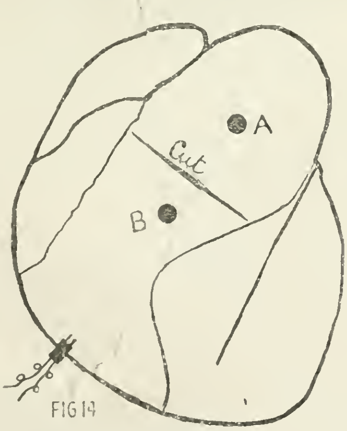


FIG 14

14.—A diagram to illustrate the method of showing that the conduction interval between two contacts is uninfluenced by cutting the superficial muscle fibre lying between them. The result is the same whether the natural or excited beat is studied. In the latter case the muscle contacts are placed in line with the stimulating electrode. Each muscle contact is paired with its own chest wall contact.

This experiment affords confirmatory evidence of the distribution through Purkinje tissue.

Section of Smaller Free Branches.—On several occasions we have successfully cut one or more of the free branches traversing the cavity of the right ventricle, using a knife edge concealed in an instrument formed like a shepherd's crook. This procedure alters the axial electrocardiogram, producing a diminution of *R* and an increase of *S*.

Muscle Section.

If a contact (fig. 14, *A*) is placed upon the conus and a cut is made below it and across the base of the conus, penetrating the muscle fibres for a few millimetres, the time at which the excitation wave appears relative to its appearance at a contact below the cut (fig. 14, *B*) is uninfluenced (see Table III).

Table III.—Natural Conus Intrinsic Deflection (from point *A*) before and after Pericardial Section.

(Readings related to intrinsic from point (*B*) below section.)

Dog.	Length of incision.	Depth.	Thickness of wall.	Intrinsic.	
				Before.	After.
H.D.	31 mm.	1–1½ mm.	3½–6 mm.	0·0092	0·0091
H.E.	22 ,,	2–3 ,,	3½–7 ,,	0·0210	0·0192
H.G.	26 ,,	2–3 ,,	4–5 ,,	0·0099	0·0105

Further, if we choose an area upon the left ventricle, where there are no conspicuou vessels, and four deep cuts are made joining each other to form a complete squar around a central contact, the appearance of the excitation wave is found to b uninfluenced by these cuts (Table IV). In performing this experiment we have to b careful to obtain the second curves as rapidly as possible after the sections ar complete, and before change in the muscle can occur from disturbance of the vascula supply.

Table IV.

Dog.	Before.	After.	Depths of cuts.	Thickness of muscle.
H.E.	0·0251	0·0240	5–7 mm.	13 mm.
H.F.	0·0223	0·0226	4–8 ,,	8–13 ,,
H.G.*	0·0159	0·0133	4–6 ,,	7–8 ,,

* In the same animal a cut parallel to the pericardial surface in another region and between t muscle layers gave delay. The readings before and after the cut were 0·0216 and 0·0285 respectively.

This experiment shows conclusively that the excitation wave spreads from within utwards, and is propagated in the ventricle without regard to the arrangement of the nuscle bands.

Rates of Propagation.

What the rate of propagation in the mammalian ventricle may be is a problem not asy to solve. It has been attempted by Gotch; he used a base-apex lead and neasured the interval between the onset and culmination of the chief deflection, onsidering this time to be the base-apex transmission interval. Now a measurement f this kind is justifiable only if it be known beforehand that the natural excitation ave has a simple spread along the muscle fibres and exactly in the line of the ontacts. Both premisses were assumed by Gotch; but, as we can clearly show, either is warranted by the facts. It is impossible to calculate and express the rate f propagation even approximately, from the times at which the natural excitation ave appears at given points on the ventricular surface, even if we have accurate stimates of these readings. The course of the excitation wave, relative to a pair of rface points, is not the same for different regions of the heart. If we use our own stimates of the surface readings in this fashion, the calculated rate varies from several undred millimetres per second to infinity. Evidently the wave may start between vo contacts. Still more important, it may not spread to any extent from one muscle ement to another, but may be separately initiated in a large number of small areas rough a special system of distribution; such is indeed our thesis.

It is necessary at the outset to study the simplest forms of propagation, forms of ropagation which are, in a measure, subject to control. For this purpose we promote tificial waves of excitation in the ventricle by stimulating it with rhythmic and reshold induction shocks at a rate in excess of the natural heart rhythm. Two ntacts are placed at a measured distance apart upon the ventricle, and contractions e forced from a point in line with them (see fig. 14). The interval between the ne-readings at the two contacts, each paired to an indifferent contact on the chest ll, gives us a measure of conduction rate over the muscle strip examined.

Influence of Heart Rate.—We made a number of preliminary observations upon the luence of heart rate. This we considered to be important, seeing that in all periments upon forced rhythms the heart rate is enhanced. Our results are shown Table V. This Table includes the intervals for natural and excited rhythm, asured alternately. We also give the distance of contacts from each other, and the tance of the proximal contact (that which first receives the excited wave) from the nt of stimulation. We find that the rate of the excited rhythm may vary widely hout producing appreciable change in the rate of propagation. There may be a le lowering of conduction rate as the heart rate enhances, but it is trifling in eriments in which rates of 170 or 200 to 300 are tested. It will be noticed in this succeeding Tables, that the interval for the natural beat is always much shorter than the interval for the excited beat (Plate 12, fig. 28); an observation which ha also been made by ERFMANN.

Influence of Fibre Direction.—We studied this question over the right ventricl (Table VI) and found higher rates in the direction of the superficial fibres than acros them, for the most part; but our results did not bring us satisfaction for the reaso that regions of the ventricle studied for straight or cross conduction necessarily varie in respect of underlying structures. Over the left ventricle, where muscle regions ca be chosen in which this factor is less disturbing, we could find little or no difference i rate (Table VII). Our results are sufficient we think to show that the direction c the superficial fibres has no material influence. But these studies brought out a fac of very considerable importance.

TABLE V.—Transmission Rates.

(Influence of Heart Rate.)

Dog.	Contacts.	Contacts apart in mm.	Natural beat.		Excited beat.			
			Heart rate.	Interval in secs.	Heart rate.	Interval in secs.	Stimulus to proximal contact in mm.	Transmission rate in mm. per sec.
G.E.	Apex to base of R.V. . .	22	206	0·0022	238	0·0145	35	1507
			184	0·0028	348	0·0182		1208
G.F.	Mid R.V. to conus . . .	27	167	0·0144	170	0·0227	21	1189
			168	0·0137	194	0·0223		1211
			164	0·0136	223	0·0239		1129
G.H.	Mid R.V. to conus . . .	17	139	0·0051	158	0·0119	17	1429
			150	0·0053	171	0·0109		1559
			147	0·0059	194	0·0111		1531
			145	0·0056	204	0·0119		1429
			146	0·0056	244	0·0109		1559
			146	0·0051	258	0·0113		1504
			143	0·0054	294	0·0109		1559
G.I.	Central region to conus .	13	214	0·0048	218	0·0078	Constant	1667
			216	0·0054	249	0·0085		1528
			214	0·0053	274	0·0088		1477
G.J.	Central region to base R.V.	28	177	0·0107	179	0·0166	Constant	1686
			172	0·0121	194	0·0163		1718
			174	0·0110	224	0·0165		1696
			170	0·0109	249	0·0169		1656
			169	0·0120	274	0·0171		1644
			152	0·0102	342	0·0173		1618
G.G.	Apex R.V. to conus . .	22	140	0·0182	185	0·0219	22	1004
			89*	0·0184	221	0·0221		996

* Heart dilating.

TABLE VI.—Transmission Rates in Right Ventricle.

Dog.	Contacts.	Contacts apart in mm.	Natural beat.		Excited beat.			
			Heart rate.	Interval in secs.	Heart rate.	Interval in secs.	Stimulus to proximal contact in mm.	Transmission rate.
	1. Leading in Direction of Fibres.							
G.F.	Mid R.V. to conus	27	168	0·0137	194	0·0223	21	1211
G.E.	Apex to base of R.V.	22	206	0·0022	238	0·0145	35	1507
G.G.	Apex R.V. to conus	22	140	0·0182	185	0·0219	22	1004
G.H.	Mid R.V. to conus	17	147	0·0059	194	0·0111	17	1531
G.I.	Central region to conus	13	216	0·0054	249	0·0085	—	1528
G.I.	Conus to conus	17	212	0·0101	282	0·0114	—	1491
G.J.	Central region to base R.V.	28	170	0·0109	249	0·0169	—	1656
G.O.	Mid R.V. to base R.V.	22	100	0·0076	184	0·0103	23	2136
G.P.	Mid R.V. to base R.V.	22	159	0·0086	182	−0·0012	15	Infinity
	2. Leading Across Fibres.							
G.O.	Central region to right margin	19	109	0·0143	189	0·0335	15	570
G.O.	Central region to right margin	19	112	0·0160	185	0·0223	34	842
G.N.	Central region to right margin	13	215	0·0081	262	0·0119	21·5	1092
G.N.	Central region to right margin	14	227	0·0074	258	0·0131	20	1068
G.M.	Mid R.V. across	13	121	0·0039	172	0·0142	15	916
G.L.	Central region to right margin	14	160	0·0102	189	0·0184	16	761
G.K.	Conus to conus	16	134	−0·0018	184	0·0119	35	1344
G.J.	Conus to conus	19	183	0·0042	206	0·0390	—	487
G.J.	Conus to conus	19	182	0·0035	212	0·0405	—	469
G.P.	Mid R.V. across	16	163	0·0001	185	0·0097	9	1679
G.R.	Across R.V.	14	169	0·0070	186	0·0157	43	891

TABLE VII.—Transmission Rates in Left Ventricle.

Dog.	Contacts apart in mm.	Natural beat.		Excited beat.			
		Heart rate.	Interval in secs.	Heart rate.	Interval in secs.	Stimulus to proximal contact in mm.	Transmission rate in mm. per sec.
	1. Leading in Direction of Fibres.						
G.N.	13	217	−0·0009	255	0·0200	13	650
G.O.	19	119	0·0017	193	0·0356	8	534
G.P.	14	158	0·0000	194	0·0241	11	581
	2. Leading Across Fibres.						
G.N.	13	204	−0·0001	265	0·0306	14	424
	13	213	−0·0051	258	0·0311	15	418
G.O.	17	113	−0·0028	198	0·0371	9	459
G.P.	22	156	−0·0050	191	0·0294	10	749

TABLE VIII.—Transmission Rates across Interventricular Groove.

Dog.	Contacts.	Contacts apart in mm.	Natural beat.		Excited beat.			
			Heart rate.	Interval in secs.	Heart rate.	Interval in secs.	Stimulus to proximal contact in mm.	Trans-mission rate.
G.F.	Aortic base to conus .	25	166	0·0241	181	0·0578	10	432
G.I.	Aortic base to conus .	23	202	−0·0018 0·0082	264	0·0379	10	607
G.I.	L.V. to mid R.V. . .	21	208	−0·0099	262	0·0235	—	894
G.I.	L.V. to apex R.V. . .	19	189	−0·0097	258	0·0239	—	795
G.J.	L.V. to central region R.V.	19	178	−0·0189	198	0·0283	—	671
G.J.	L.V. to central region R.V.	37	179	0·0091	202	0·0771	—	480
G.K.	Trabeculated area to mid conus	22	128	0·0080	180	0·0623	15	333
G.K.	Mid L.V. to mid R.V.	22	124	−0·0174	177	0·0570	20	386
G.L.	Trabeculated area to R.V.	23	158	−0·0050	186	0·0509 0·0587	10	452 392
G.M.	Trabeculated area to R.V.	22	115	−0·0072	174	0·0529	18	416
G.M.	L.V. to mid R.V. . .	24	112	−0·0082	182	0·0567	9	423
G.N.	L.V. to mid R.V. . .	17	225	−0·0133	242	0·0416	8	409
G.N.	L.V. to trabeculated area	14	211	−0·0112	248	0·0186	15	754
G.O.	L.V. to mid R.V. . .	17	112	−0·0192	188	0·0271	8	627
G.O.	L.V. to trabeculated area	10	112	−0·0118	189	0·0200	8	500

Influence of Muscle Thickness.—Excited waves are propagated much more slowl
over the surface of the left ventricle than over the surface of the right, an
conduction is also invariably slow across the interventricular groove (Table VIII)
The calculated rates for the right ventricle vary usually between 1000 and 1500 mm
per second (Plate 12, fig. 28). Those for the left ventricle vary usually betwee
400–600 mm. per second; the rates over the interventricular groove (Plate 12
fig. 29) and between points on and off the trabeculated region are of simila
magnitude. There is a distinct and constant variation of rate over certain areas
the heart which we can attribute only to the constitution of the deeper structure
Our view is that the conduction rate in muscle is slow, and in the Purkinje syste
fast; and that when the surface of the heart is stimulated, the excitation wav
penetrates the muscle and enters the Purkinje network. When the wall examine
is thin, conduction will be more through the Purkinje substance and less throug
muscle; when the wall is thick, the reverse will be the case; so in the one instan
conduction will be more rapid and in the other instance less rapid. Such at
events was the hypothesis which we determined further to examine. If t
conditions are as we imagine them to be, and two contacts are placed on the rig

ventricle a considerable distance from the point of stimulation, the one contact on the outer surface and the other upon the inner or endocardial surface, we should expect to find that the internal contact is the first to receive the excitation. Such proves to be the case. Now, when the heart beats naturally, activity is always signalled by an internal contact before it is signalled by an external contact placed immediately over it. When the heart is stimulated at a surface point some distance from such contacts, not only does the endocardial surface receive the excitation wave before the epicardial surface, *but it does so by precisely the same time interval as during the natural heart beat* (Table IX, Plate 12, fig. 30, italicised figures). This statement is only true, however, when the point of stimulation is sufficiently distant. The muscle surface is stimulated and the excitation wave spreads by two routes. It spreads over the superficial muscle towards the external contacts (path *aa* in fig. 15), it penetrates the muscle, enters the Purkinje substance and reaches the internal contact, to penetrate the muscle once more and reach the external contact (path *bb* in fig. 15). But the distance of the stimulating electrode from the contacts is of prime importance. If it is but a few millimetres, the path *aa* will be as quick as the other or quicker. By measuring the thickness of muscle and the distance between stimulating electrodes and contacts, we may arrive at certain conclusions in respect of the relative rates of conduction. Take, for example, the last experiment of Table IX. The muscle is 6 mm. in thickness and the distance 15 mm. The path through 6 mm. muscle + 15 mm. Purkinje + 6 mm. muscle is preferred to the path through 15 mm. muscle; or, briefly, 15 mm. of Purkinje are preferred to 3 mm. of muscle. We have clear evidence from this and other examples that the rate of travel through Purkinje substance is much faster than through muscle. We calculate

TABLE IX.—Transmission from Internal to External Contacts.

Dog.	Contacts.	Contacts apart in mm.	Natural beat.		Excited beat.			Natural transmission rate (mm. per sec.).
			Heart rate.	Interval (internal to external).	Heart rate.	Interval (internal to external).	Stimulus to external contact in mm.	
G.L.	Base of R.V. internal to external	4	115 115	*0·0095* 0·0092	215 185	*0·0098* 0·0071	*32* 10	421
G.N.	Base of R.V. internal to external	4	113 113	*0·0113* 0·0113	172 176	*0·0109* 0·0026	*18* 10	354
G.O.	Base of R.V. internal to external	6	112 113 109	*0·0205* *0·0207* 0·0204	192 188 193	*0·0226* *0·0227* 0·0170	15* 15† 8†	293

* Stronger stimulus.

† Threshold stimulus.

that it is at least three to five times as fast, regarding the Purkinje tissue not as a network, but as a straight band of fibres.

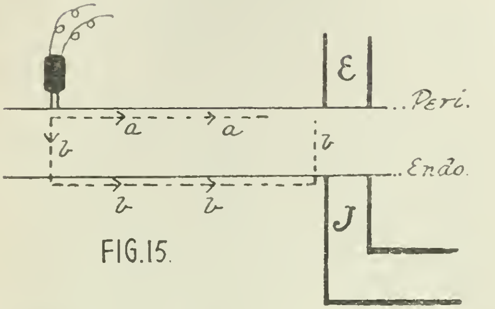


FIG. 15.

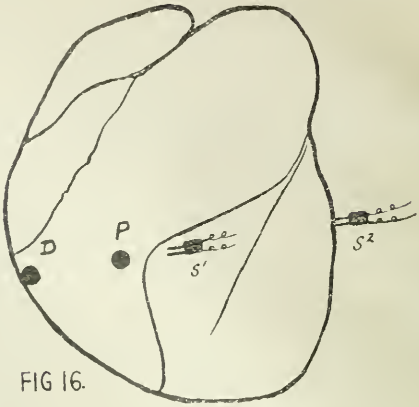


FIG 16.

FIG. 15.—A diagram showing the ventricular wall in section and the paths travelled by the excitation wave, from the point of stimulation to the external and internal contacts.

FIG. 16.—A diagram illustrating an arrangement of contacts, with the stimulating electrodes placed near to, and far from, the proximal contact. When the ventricle is excited at S^2 the interval between the appearance of the excitation wave at P and D is shortened.

The distance of the point of stimulation from the contacts shows its influence in an experiment of a somewhat different type. Two contacts (*P* and *D*) are placed across the right ventricle (fig. 16), and a point of stimulation is chosen a little to the left (lying usually over the trabeculated region or near its border) and in line with them (S^1). The time interval between the contacts is ascertained for natural and excited beats; the latter, as usual, exceeds the former. The stimulating electrodes are now moved along the same line to the left border of the left ventricle (S^2) or towards it, thus increasing their distance from the proximal contact (*P*). The time interval between the two contacts is invariably reduced by this procedure; and the reduction, more often than not, is such that the interval for excited and natural beats becomes the same (Table X, examples marked with asterisks). Our explanation is that the excitation wave, instead of taking the shorter but slower path through the muscle of the septum, prefers the longer but faster path through the left division of the bundle over the septum and down the right division to the muscle underlying the contacts. The race along the two paths is often a close one, and consequently the reduction is not always so extensive; but in these circumstances the new value for the excited beat lies between its former value (when the stimulating electrodes are close up) and its value for the natural beat. That the endocardial surface, and presumably the Purkinje network, is followed when an excitation wave spreads from a point of stimulation can be shown quite clearly. We place two contacts on the right ventricle and stimulate at a point some distance away but in line with them (fig. 14). The time interval between the appearance of the excitation wave at the two contacts is established, and a long incision is then made between them and at right angles to them. The incision penetrating the superficial muscle fibres for one-third or one-half their thickness does not

TABLE X.—Transmission Rates.

(Influence of distance of stimulation point.)

Dog.	Contacts.	Contacts apart in mm.	Natural beat.		Excited beat.			
			Heart rate.	Interval in secs.	Heart rate.	Interval in secs.	Stimulus to proximal contact in mm.	Transmission rate in mm. per sec.
G.K.	Central region to margin R.V.	17	128	0·0060*	182	*0·0066**	*41*	*2758*
G.L.	Central region to margin R.V.	14	164	0·0087*	199	*0·0104**	*38*	*1346*
			160	0·0102*	189	0·0184	16	761
G.M.	R.V. across	15	137	0·0044*	169	*0·0042**	*42*	*3571*
		13	121	0·0039	172	0·0142	15	916
G.N.	,, ...	14	227	0·0070*	242	*0·0092**	*42*	*1522*
			227	0·0074	258	0·0131	20	1068
G.O.	Mid R.V. to base R.V. .	19	112	0·0160	185	*0·0223*	*34*	*857*
			109	0·0143	189	0·0335	15	570
G.P.	,, ,, .	16	167	-0·0008	190	*0·0036*	40	*4444*
			163	0·0001	185	0·0097	16	1650
G.R.	,, ,, .	14	169	0·0070	186	*0·0157*	*43*	*892*
			173	0·0045	184	0·0266	13	527
G.R.	,, ,, .	11	173	0·0091*	184	*0·0087**	*40*	*1264*
			169	0·0072	179	0·0216	11	509
G.Z.	R.V. across	17	115	0·0066*	195	*0·0065**	*45*	*2461*
			115	0·0061	198	0·0302	17	530

* Note the approximation of these readings in the columns referring to natural and excited beats.

influence conduction in the least (Table XI). The wave is conveyed across the incision just as readily as it was conveyed across the same region in its undamaged state.

TABLE XI.—Excited Conus Intrinsic Deflection before and after Pericardial Section.

(Readings related to intrinsic from conus point below section.)

Dog.	Length of incision.	Depth.	Thickness of wall.	Intrinsic.	
				Before.	After.
H.D.	31 mm.	1–1½ mm.	3½–6 mm.	0·0208	0·0192
H.E.	22 ,,	2–3 ,,	3½–7 ,,	0·0143	0·0143
H.G.	26 ,,	2–3 ,,	4–5 ,,	0·0177	0·0160

Clearly this can occur only if the original course was a deep one. So it happens that if a similar cut is made on the endocardial surface the excited wave is interfered with, the time interval increasing conspicuously (Table XII).

TABLE XII.—Excited Conus Intrinsic Deflection before and after Endocardial Section (Readings related to intrinsic from conus point below section.)

Dog.	Length of incision.	Depth.	Thickness of wall.	Intrinsic.	
				Before.	After.
H.D.	31 mm.	1½–4 mm.	4–5 mm.	0·0026	0·0126
H.E.	27 ,,	0–3 ,,	5–6 ,,	0·0112	0·0234
H.F.	Endocardium scratched in places over 15 mm.		2–5 ,,	0·0104	0·0247
H.G.	Endocardium scratched over 20 mm.		5 ,,	0·0057	0·0274

Actual Rates of Propagation.

To speak of the rate of propagation in the ventricular wall would bring us no nearer the essential problem. We have, however, to discuss the actual rates of propagation in its two elements, ventricular muscle and Purkinje substance. The high rates of conduction along the thin wall of the right ventricle are due according to our view to escape into the Purkinje tissue, the low rates along the thicker muscle of the left side to the relatively lower powers of conduction in the muscle itself. Seeing that there is no reason to believe that the rate of conduction in the muscle is variable in different regions, or that there is appreciable variation according to the direction of the superficial fibres, we ascribe all the chief variations in rate to early or late escape to the Purkinje network. Holding this view we regard the lowest conduction rates obtained, *i.e.*, those approaching 300 or 500 mm. per second, as representing the real ventricular rate most closely; while we regard the highest rates, *i.e.*, 1500 or 2000 mm. per second, as low estimates* of the network rate. These estimates would suggest that network conduction is approximately five times as fast as muscle conduction; an estimate in remarkably close accord to a previous one.

But network conduction does not fully represent Purkinje conduction, for the fibres of the network are not straight, but are, for the most part, very wavy. Conduction in such strands as contain straight fibres, *i.e.*, the chief divisions of the bundle, should be 50 or 100 per cent. faster.

Our estimate for Purkinje substance as such is from 3000 to 5000 mm. per second and we favour the last figure rather than the first.†

---

* Low because these calculations are for Purkinje substance with two short stretches of *muscle* added.

† It seems clear from our experiments that if an excitation is artificially propagated from the ventricular surface this wave rapidly finds its way into the Purkinje system. It is also evident

Our estimate of conduction rates in the three tissues of the heart which are concerned may be stated as follows :—

Purkinje tissue	3000–5000 mm. per sec.
Auricular muscle	1000 mm. per sec.
Ventricular muscle	300–500 mm. per sec.

In harmony with these estimates is the fact that by far the highest glycogen content of the heart is to be found in Purkinje tissue (Nagayo) while auricular muscle is stated by Berblinger* to be more heavily laden than ventricular. The functional significance of these differences in the power of conduction will be alluded to a little later.

The Endocardial Lining.

Observations upon the endocardial surface of the ventricle present many natural difficulties. We have employed many different forms of electrode; the most satisfactory results are obtained with single and rigid contacts of glass tubing curved to suit the point or points examined. The glass tube has a broad outlet filled by a kaolin plug; the shaft of the tube contains copper sulphate and a copper wire, the opening and an inch or more of the shaft being sealed with hard paraffin wax. The contact is introduced through an auricular appendix and paired with a second contact upon the chest wall. In one or two experiments closely set double contacts were used internally, but these proved less satisfactory. The introduction of an internal electrode disturbs the position of the heart to some extent and in unfortunate experiments may lead to serious disturbance of mechanism.

We reject all observations in which the normal form of axial electrocardiogram is not maintained. All internal leads are undertaken as the final observations of a given experiment. Most of our readings have been taken from the lining of the free wall of the right ventricle, and these are the only observations which we regard with entire confidence. In studying this region with a rigid curved electrode the thin muscular wall may be lifted a little on the contact, so that it bulges externally and so that the contact may be easily felt through the wall and its exact position ascertained and maintained. In the circumstances there is no uncertainty, the contact is known to be satisfactory. But when we attempt to obtain readings from the septum on the

---

that the rate of propagation in the system is very rapid. When an idio-ventricular rhythm dominates the movements of the ventricle and premature contractions are forced from the ventricle, the length of the returning cycle (see first article) is little if at all longer than an initial or natural cycle. This fact has never received adequate explanation. It now seems that the time lost by the excitation wave in travelling from the point stimulated to the rhythm centre in the auriculo-ventricular bundle would not be appreciable. If the natural ventricular cycle has a duration of a second or more, an addition of two or three hundredths of a second would pass unnoticed.

[*] 'Beiträge z. pathol. Anat. u. allg. Pathol.,' vol. 53, p. 207 (1912).

right side, and especially when we introduce the electrode into the left chamber, accurate location is almost impossible.*

The power of the left ventricle is so great and the wall so rigid that the electrode may be unmanageable and the fling very considerable. Consequently we lay most stress upon readings from the thin and free wall of the right chamber.

Internal and External Readings.

If we place one electrode upon the inner and the other upon the outer surface immediately over it, so that the two contacts grip the muscle lightly between them; then the internal contact invariably receives the excitation current first. This fact is clearly shown by Table XIII. The interval between the two readings (internal and external) is not the same for different regions of the wall. It is greatest over the base near the auriculo-ventricular groove, where the muscle is thick, and least in the central region where the muscle is thinnest. If we measure this muscle thickness and calculate the rate of propagation on the basis of the corresponding internal-external interval (Table IX) we find it to be about 300–400 mm. per second, values which harmonise

TABLE XIII.—Comparison of Internal and External Leads.
(Appearance of extrinsic and intrinsic deflections relative to *R* in Lead *II*.)

Dog.	Extrinsic.		Intrinsic.		Contacts.*
	Internal.	External.	Internal.	External.	
G.A.	None	−0·0033	−0·0015	0·0131	On anterior papillary muscle and over same.
G.F.	None	−0·0108	−0·0082	0·0088	On anterior papillary muscle and over same.
G.J.	−0·0101	−0·0104	−0·0023	0·0082	Base of R.V. and over same.
	−0·0098	−0·0110	−0·0013	0·0020	Apex of R.V. and over same.
G.K.	−0·0068	−0·0056	−0·0032	0·0172	Base of R.V. and over same.
	−0·0059	−0·0067	−0·0046	0·0069	Central region and over same.
G.L.	−0·0077	−0·0048	−0·0005	0·0147	Base of R.V. and over same.
	−0·0120	—	−0·0010	0·0049	Central region and over same.
G.M.	−0·0110	−0·0087	−0·0022	0·0102	Mid R.V. and over same.
	−0·0068	−0·0069	−0·0022	0·0100	Base of R.V. and over same.
H.C.	—	—	−0·0001	0·0213	Base of R.V. and over same.
	−0·0118	−0·0073	−0·0029	0·0094	Apex of R.V. and over same.
	−0·0109	−0·0073	−0·0020	0·0035	Central region and over same.

* With the exception of the first two experiments, in which double contacts were used, each contact named was paired with a chest wall contact.

---

* We are helped in the location of such contacts by running a long pin through the heart near the contact while the electrode is *in situ*.

with our former calculation of the rate in ventricular muscle. We consider therefore that the time of appearance of the excitation wave at the external contact is governed largely by the thickness of muscle underlying it. Eventually this subject will be discussed more fully.

In Table XIV we have summarised all our endocardial readings, and may briefly consider them from the regional standpoint.

Central Region.—There are 6 readings (0·0046, −0·0010, −0·0030, −0·0002, −0·0026 and −0·0020) from this region.

The endocardial surface becomes excited as a general rule a little before *R* has its onset. The figures cannot strictly be compared with each other; it is necessary to take into account the external surface distribution in each heart; for this reason we

TABLE XIV.—Deflections from Internal Contacts.

Dog.	Contact.	Intrinsic.	Extrinsic.	Q.	Earliest external intrinsic.	Earliest external extrinsic.
G.F.	R.V. anterior papillary muscle	−0·0082	Absent*	−0·0058	−0·0031	−0·0111
	Same a little higher	−0·0072	Absent*			
G.J.	R.V. base	−0·0023	−0·0101	−0·0058	−0·0029	−0·0121
	R.V. apex	−0·0013	−0·0098			
G.K.	R.V. base	0·0032	−0·0068	−0·0053	0·0037	−0·0077
	R.V. central region	0·0046	−0·0059			
G.L.	R.V. base	−0·0005	−0·0077	−0·0096	0·0021	−0·0161
	R.V. central region	−0·0010	−0·0120			
	Same (a little higher)	0·0002	−0·0166			
G.M.	R.V. mid	−0·0022	−0·0110	−0·0048	0·0000	−0·0077
	R.V. anterior papillary muscle	−0·0008	−0·0203 ?			
	R.V. mid-septum	−0·0033	−0·0075			
	R.V. base	−0·0022	−0·0068			
G.C.	R.V. high septal	0·0243	—*	−0·0063		
G.T.	R.V. mid	−0·0025	−0·0070	−0·0081	−0·0005	−0·0080
G.U.	L.V. apex	0·0110	−0·0077	−0·0105	−0·0001	−0·0132
	L.V. mid-septal	−0·0036	−0·0139			
G.V.	L.V. apex	−0·0019	−0·0100	—	—	−0·0096
	L.V. mid-septal	−0·0085	—			
G.W.	R.V. apex	−0·0041	−0·0172	−0·0107	−0·0048	−0·0160
	R.V. central region	−0·0030	−0·0087			
	R.V. base	−0·0016	−0·0134			
	R.V. high septal	0·0144	−0·0075			
	R.V. conus	0·0038	−0·0150			
	L.V. high septal	−0·0068	−0·0192			

* Double contacts.

TABLE XIV—*continued.*

Dog.	Contact.	Intrinsic.	Extrinsic.	Q.	Earliest external intrinsic.	Earliest external extrinsic.
G.Z.	R.V. apex	0·0003	−0·0078	−0·0048 (approx.)	0·0024	−0·0086
	R.V. base	0·0009	−0·0086			
	R.V. conus (high) .	0·0025	−0·0070			
	R.V. central region .	−0·0002	−0·0078			
G.X.	R.V. apex	−0·0003	−0·0090	−0·0082	−0·0033	−0·0138
	R.V. conus	0·0202	−0·0089			
	R.V. base	0·0138	−0·0100			
G.Y.	L.V. apex	−0·0027	−0·0142	−0·0066	0·0028	−0·0156
	L.V. mid-septum . .	0·0066	—			
	L.V. high septum .	0·0022	—			
H.B.	R.V. apex	0·0031	−0·0088	−0·0041	−0·0010	−0·0099
	R.V. base	0·0018	−0·0084			
	R.V. central region .	−0·0026	−0·0094			
	R.V. papillary muscle	−0·0039	−0·0115			
	R.V. mid-septum . .	0·0060	−0·0098			
	R.V. conus	0·0092	−0·0057			
	R.V. conus (higher) .	0·0127	−0·0035			
H.C.	R.V. apex	0·0029	−0·0118	None	−0·0038	−0·0083
	R.V. base	−0·0001	—			
	R.V. central region .	−0·0020	−0·0109			
H.D.	L.V. apex	0·0031	—	—	—	—

include a column in our Table showing the earliest external reading obtained over the central region. In the case of the relatively high figure + 0·0046 which stands amongst internal readings, it is to be noted that the corresponding external readings were also higher than usual.

Apex of Right Ventricle.—From this region there are six readings (−0·0013, −0·0041, 0·0003, −0·0003, 0·0031 and 0·0029). The readings are of similar order to those of the central region. Despite this fact, the epicardial surface of the apical region reads higher than that of the central region; this is to be accounted for by the greater thickness of muscle beneath the former.

The Base of the Right Ventricle.—There are nine readings from this region (−0·0023, 0·0032, −0·0005, −0·0022, −0·0016, 0·0009, 0·0138, 0·0018, −0·0001) and two from an area between this and the central region (−0·0022 and −0·0025). The base readings are higher than those of the central region, but not so high as might have been anticipated from the distribution of the Purkinje system to this region. One figure 0·0138 is exceptionally high; we do not regard it as representative, and view it with some suspicion; it is possible that the corresponding contact was placed too near the base.

The Conus Region.—There are five readings (0·0038, 0·0025, 0·0202, 0·0092 and 0·0127). We have one exceptionally high reading in this series too; the remainder being tolerably uniform. Two of the readings (0·0092 and 0·0127) were taken from the same animal, the last (0·0127) being obtained from a contact immediately beneath the pulmonary valves. The Purkinje path to the conus is the longest of any in the right ventricle; relatively high figures were therefore to be anticipated. The muscle of the conus is as a rule no thicker than that at the base of the right ventricle; the high external readings over the conus, as compared to the base, are consequently to be attributed to the relations of the conducting system to these two portions of the wall.

We pass to less satisfactory observations upon other internal surfaces.

The Papillary Muscle.—We have attempted to obtain readings from the large muscle in the right ventricle, marking our contact point by transfixing the heart. Three contacts seemed to us to have been successfully placed and the readings are relatively low (−0·0082, −0·0008 and −0·0039). The papillary muscle seems to be one of the earliest points to become active in the right ventricle; the intrinsic deflection distinctly preceding the onset of *R*. *R* has been attributed by NICOLAI to activity of this muscle; our results do not support this view.

Right Side of Septum.—Our readings are variable, −0·0033 and 0·0060 for the mid-septal region, and 0·0243 and 0·0144 for the high septal region. These last two readings are very high, but not incompatible with the anatomical conditions. It will be remembered that Purkinje fibres in the neighbourhood of the septal cusp of the tricuspid valve are apparently sparse or absent, and this is the region upon which the contacts lay.

Apex of Left Ventricle.—Of four readings (0·0110, −0·0019, −0·0027, 0·0031) one is exceptionally high and we are at a loss to explain it. It should be remembered that readings are obtained after the experiment has terminated and that we do not become aware of such exceptions until it is too late to repeat the observations. We must accept them therefore for what they are worth, though we cannot but suspect that from time to time some unnoticed factor has crept in. Single high readings have also been noted from the base and conus of the right ventricle. We have attempted to ascertain the significance of these occasional high readings without success. When an electrode is introduced into a heart cavity, it is in contact with the whole endocardial lining through the blood; but that this introduces no appreciable error is clear from a specially devised experiment. We place a contact upon the outer surface of the heart, and estimate the time for this point before and after flooding the pericardium, slung as a hammock, with blood. The two readings are very similar (Table XV).

We thought that possibly contacts on the endocardial lining might have been completed only at the commencement of systolic contraction in certain instances.

TABLE XV.—Deflections before and after Flooding Pericardium.

Dog.	Region tested.	Extrinsic.	Intrinsic.
H.B.	L.V. near apex	−0·0070 −0·0067	0·0192 Before. 0·0168 After.
H.C.	R.V. near apex	−0·0076 −0·0056	0·0137 Before. 0·0177 After.

To test the effect of a contact jumping in this fashion, we arranged an electrode on the external surface, so that contact was made in systole only, the contact being covered with free blood in the pericardium. Subsequently we took a curve from the nearest ventricular point with the contact well maintained. The result threw no light upon our exceptional values (Table XVI).

TABLE XVI.

Dog.	Contact.	Extrinsic.	Intrinsic.
H.B.	Jumping Constant	−0·0048 −0·0067	0·0102 0·0091
H.C.	Jumping Constant	−0·0091 −0·0073	0·0022 0·0009

As a matter of fact a simple blood-contact in the centre of the right ventricular cavity gives a low value (Dog G.Z., 0·0011; Dog H.B., −0·0027).*

Left Lining of the Septum.—These contacts are the most precarious of any. Of three readings from the mid-septal region (−0·0036, −0·0085, 0·0066), two were low values, the third relatively high. Small contacts introduced through the aorta to test the region immediately beneath the aortic valves gave, in two animals, readings of −0·0168 and 0·0022 respectively. The former is the lowest reading we have obtained from any part of the heart. This is of interest, since from the distribution of the Purkinje substance this is the region where we should expect earliest activity. Directly below the valves the left division of the bundle appears (Plate 9, fig. 22), and the rebranching begins almost immediately.

But there are small areas close at hand which appear to have little or no supply. Very low values and considerably higher values would be anticipated in this neighbourhood.

---

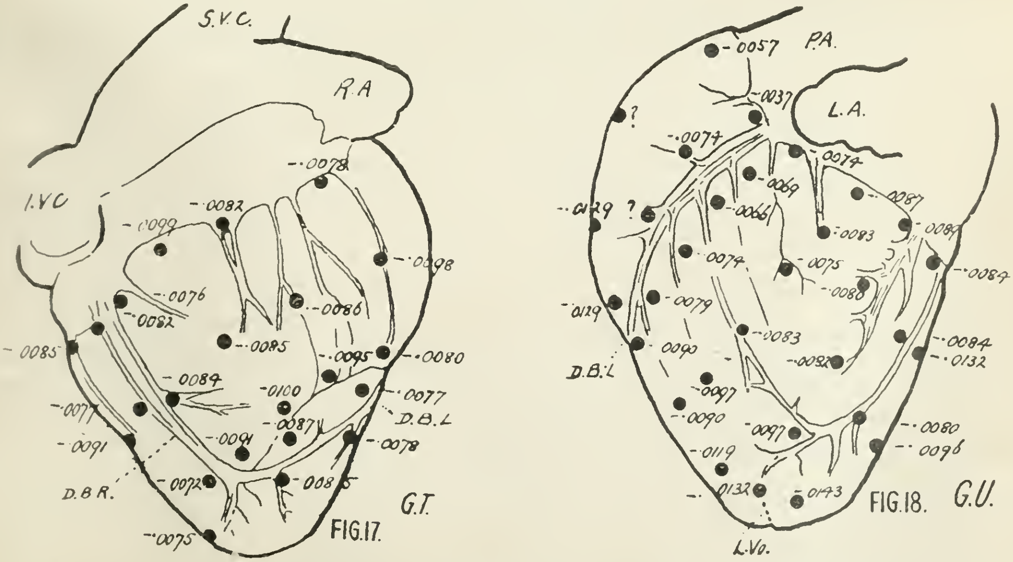
* It has since occurred to us that the exceptional high reading may be brought about by an electrode rubbing and damaging an area of the network surrounding the contact. In such a case the reading would be too high because conduction to the contact would be through muscle for some distance. The Purkinje substance is very susceptible to pressure, witness the effect of lightly clamping the *A–V* bundle.

The Endocardial Lining as a Whole.

Two facts of chief importance seem to be established. (1) The lining of the ventricular wall is always activated before the pericardial surface, and our results show that the interval between the internal and external activity is controlled by the thickness of muscle at the corresponding point. A simple muscular spread of the excitation wave from within outwards, with uninterrupted penetration of the several muscle layers accounts for the phenomena observed. (2) The internal surface is supplied at a very early period of the ventricular cycle, and at a number of points activity appears almost simultaneously. The earliest region to be activated, of which we can speak positively from direct observation, is the area supplied by the early outgoing branches of the right bundle division; other regions, as will presently be understood, are even earlier, and it is suspected, both from anatomical studies and from solitary direct leads, that the lining of the left side of the septum is in reality the first to be involved in the ventricle as a whole. The conus is a region in which we can speak definitely of a late arrival of the excitation wave.

The Earliest Appearance of the Excitation Wave.

The earliest sign of the ventricular excitation wave is not to be found in *R* in axial leads. This is preceded in the majority of electro-cardiograms by a small depression *Q*, which is unquestionably ventricular in origin. The reading for *Q* in respect of *R* is, as a rule, between −0·0050 and −0·0100 second. But the earliest sign is to be found in extrinsic deflections. In figs. 6 and 9, a number of observations upon ntrinsic deflections are charted. The earliest extrinsic deflections, over the same contact points are charted in figs. 17 and 18, and the extrinsic deflections corresponding to other figures are tabulated (Tables XVII and XVIII).



IGS. 17 and 18.—The extrinsic deflections at the contacts over the right ventricle (Dog G.T.) and over the left ventricle (G.U.). The observations are from the same hearts as those shown in figs. 6 and 9.

TABLE XVII.—Dog G.L.

Contact.		Extrinsic.	Intrinsic.
1	R.V.	−0·0025	0·0151
2		−0·0046	0·0241
3		−0·0045	0·0261
4		−0·0061	0·0231
5		−0·0050	0·0198
6		−0·0059	0·0169
7		−0·0038	0·0150
8		−0·0076	0·0049
9		−0·0078	0·0082
10		−0·0059	0·0033
11		−0·0053	0·0167
12		−0·0066	0·0100
13		−0·0063	0·0021
14		−0·0028	0·0073
15		−0·0057	0·0178
16		−0·0069	0·0014
17		−0·0080	0·0054
18		−0·0053	0·0073
19	L.V.	−0·0063	0·0195
20		−0·0042	0·0213
21		−0·0052	0·0203
22		−0·0020	0·0146
23		−0·0013	0·0187
24		−0·0000	0·0196
25		−0·0044	0·0141

Q = −0·0096.

TABLE XVII.—Dog G.M.

Plate and contact.	Extrinsic.	Intrinsic.
1	−0·0077	0·0146
2	−0·0048	0·0212
3	−0·0054	0·0231
4	−0·0053	0·0266
5	−0·0039	absent
6	−0·0057	absent
7	−0·0052	0·0204
8	−0·0033	0·0222
9	−0·0040	0·0211
10	−0·0069	0·0160
11	−0·0070	0·0129
12	−0·0059	0·0187
13	−0·0069	0·0000
14	−0·0061	0·0027
15	−0·0036	0·0185
16	−0·0042	0·0075
17	−0·0069	0·0029
18	−0·0044	0·0183
19	−0·0051	0·0084
20	−0·0053	0·0133
21	−0·0035	0·0235
22	−0·0049	0·0083
23	−0·0043	0·0049
24	−0·0044	0·0165
25	−0·0053	0·0155
26	−0·0059	0·0157
27	−0·0063	0·0149
28	−0·0039	0·0188
29	−0·0058	0·0190

Q = −0·0048.

TABLE XVIII.—Dog G.W.

Plates and contact.		Extrinsic.	Intrinsic.
1		−0·0110	0·0058
2		−0·0087	0·0081
3		−0·0069	0·0140
4		−0·0093	0·0145
5		−0·0084	0·0256
6		−0·0082	0·0237
7		−0·0152	0·0241
8		−0·0149	0·0003
9		−0·0133	0·0050
10		−0·0114	0·0058
11		−0·0080	0·0226
12		−0·0102	0·0177
13		−0·0133	0·0038
14		−0·0113	−0·0029
15		−0·0125	−0·0048
16		−0·0135	−0·0013
17		−0·0149	0·0166
18		−0·0160	0·0139
19		−0·0158	0·0144
20	Internal contacts.	−0·0172	−0·0041
21		−0·0087	−0·0030
22		−0·0134	−0·0016
23		−0·0075	0·0144
24		−0·0150	0·0038
25		−0·0192	−0·0168

Q = −0·0107.

When we lead from a pair of contacts one of which lies on the heart and the other on the chest wall, the times at which extrinsic deflections appear are, with a few exceptions, wonderfully uniform. In fig. 17 (G.T.) the values range from −0·0072 to −0·0100; in fig. 18 (G.U.) the majority of the readings range from −0·0037 to −0·0119, though a few readings are lower. The average of readings usually approximates to the value for *Q* but there are always some extrinsic deflections which precede *Q*.

That the extrinsic deflection results from the excitation of some deep lying mass of muscle cannot be doubted; that most extrinsic deflections from a single heart represent the activation of the same region of that heart seems clear from the general uniformity of the readings. It is certain that the earliest extrinsic deflection from a given contact is not an expression of activity in the deeper layers of the muscle wall at the point investigated, for, if that were so, the values should rise over the right ventricle wher the right branch division is transected. In point of fact they do not rise (Table XIX) It seems clear, therefore, that extrinsic deflections recorded from a contact over the ight ventricle may represent the passage of an excitation wave through portions of he left ventricle.

TABLE XIX.—Deflections before and after Section of Bundle Branch.

Before section.			After section.		
Plate.	Extrinsic.	Intrinsic.	Plate.	Extrinsic.	Intrinsic.
		Dog G.P. (see fig. 12).			
9 (L.V.)	−0·0071	0·0179	18	−0·0102	0·0154
10 (L.V.)	−0·0085	0·0217	19	−0·0083	0·0206
11 (T.A.)	−0·0098	0·0081	20	−0·0116	0·0294
12 (R.V.)	−0·0098	0·0154	21	−0·0093	0·0544
13 (R.V.)	−0·0085	0·0148	22	−0·0090	0·0590
14 (L.V.)	−0·0060	0·0127	23	−0·0122	0·0114
15 (L.V.)	−0·0081	0·0168	24	−0·0103	0·0146
16 (R.V.)	−0·0074	0·0149	25	−0·0112	0·0390
17 (R.V.)	−0·0086	0·0251	26	−0·0088	0·0624
		Dog G.R. (see fig. 13).			
6 (R.V.)	−0·0050	0·0157	16	−0·0069	0·0516
7 (R.V.)	−0·0058	0·0027	17	−0·0089	0·0439
8 (T.A)	−0·0089	0·0148	18	−0·0083	0·0300
9 (L.V.)	−0·0048	0·0265	19	−0·0048	0·0229
10 (L.V.)	−0·0050	0·0283	20	−0·0054	0·0219
11 (L.V.)	−0·0083	0·0282	21	−0·0064	0·0241
12 (L.V.)	−0·0031	0·0285	22	−0·0016	0·0262
13 (T.A.)	−0·0056	0·0127	23	−0·0058	0·0243
14 (R.V.)	−0·0023	0·0182	24	−0·0044	0·0520
15 (R.V.)	−0·0050	0·0252	25	—	0·0675

The earliest sign of activity in the ventricle as a whole, as estimated from the values f extrinsic deflections, comes between 0·0070 and 0·0192 second before the upstroke f *R*. When we were studying the auricle we found that the value of the earliest xtrinsic deflection practically coincided with the value of the earliest intrinsic deflection in this chamber as a whole. In the auricle this fact can be easily established ecause the excitation wave spreads from a single centre, whose position is favourable o observation.

In the ventricle our earliest extrinsic deflections clearly indicate that some region s activated as a rule 0·0100 or 0·0150 second before *R* appears (and 0·0050 second r more before *Q* is written). We had hoped to find intrinsic deflections of like value, ut in this we have been disappointed. Sometimes our earliest intrinsic would pproach to within 3, 4 or 6 thousandths of a second of the earliest extrinsic eflection; in other instances a hiatus of a hundredth of a second or a little more would e left (see Table XIV). That there is generally a hiatus is perhaps not surprising, eeing that many readings cannot be secured from the lining of the heart; our view hat the excitation wave appears first on the left side is strongly supported by those experiments in which a number of points on the right endocardial surface have been investigated. In none of these has that area been discovered which is responsible for the earliest extrinsic deflection. Were it in the right heart we believe it could hardly escape detection.

Actual and Calculated Readings.

Our hypothesis is that the time at which the excitation wave appears at any point on the surface of the ventricle is controlled by two chief factors; firstly, by the length of the Purkinje tract from its starting point to the network immediately beneath the point in question; and, secondly, by the thickness of the ventricular wall. In two experiments we have attempted to make these measurements. In measuring the length of the Purkinje tract we place a piece of cotton upon the inner surface of the ventricle, so that it overlies what we judge to be the shortest Purkinje path, from the division of the main bundle to that part of the endocardium which is nearest to our marked point on the epicardium. The cotton is subsequently measured in millimetres. In estimating the thickness of muscle we do not always measure vertically to the surface, for frequently an oblique path to the nearest endocardial depression is the shorter one. We measure the heart after hardening in the distended position with formalin; and calculate how long the excitation wave will take to travel along so many millimetres of Purkinje substance, on the basis of a rate of 5000 mm. per second; we use 500 mm. per second as a basis for the rate of propagation in the muscle. In this way we arrive at the total time for the appearance of the surface excitation wave. To express this calculated value in relation to *R* we must subtract the interval between the commencing distribution in the bundle branches and the appearance of *R*: our closest estimate of this interval is the time which elapses between the earliest extrinsic deflection and *R*.

Thus if the Purkinje measurement is 51 mm., we allow 0·0102 second; if the muscle thickness is 4 mm., we allow an additional 0·080 second = 0·0182 second. If the earliest extrinsic comes 0·0100 second before *R*, our final estimate stands at + 0·0082.

We publish our two experiments, with the corresponding calculated values, in Tables XX and XXI. In one instance the left ventricle, in the other the right ventricle, has been utilised. Considering the many possible sources of error in measurement, and remembering that here and there at infrequent intervals twigs of the Purkinje network penetrate the muscle for a few millimetres, the calculated values approximate to the actual values in a most satisfactory manner. Especially is this the case in the left ventricular Table. The maximal divergence in both Tables is less than a hundredth of a second; the average divergence is but a few thousandths of a second.

But for our argument we are content to point out that the actual and calculated *orders* of the points bear far more than a coincidental relation to each other; and tha by calculation alone we may obtain a large series of surface values which gives a very accurate general survey of the actual distribution of the excitation wave.

TABLE XX.—Actual and Calculated Intrinsic Deflections.
(Right Ventricle, Dog G.X.)

Muscle thickness in mm.	Purkinje pathway in mm.	Calculated intrinsic.	Actual intrinsic.	Actual extrinsic.	Order of contact.
1·0	45	−0·0028	−0·0033	−0·0073	12
1·75	50	−0·0003	−0·0018	−0·0111	11
2·0	65	0·0032	0·0030	−0·0094	10
2·5	67	0·0046	0·0090	−0·0066	14
3·0	75	0·0072	0·0166	−0·0102	9
3·0	56	0·0034	0·0009	−0·0100	6
3·0	73	0·0068	−0·0012	−0·0117	7
3·5	45	0·0022	0·0091	−0·0083	13
4·0	60	0·0062	0·0064	−0·0088	5
4·5	67	0·0086	0·0129	−0·0138	8
5·0	73	0·0108	0·0183	−0·0079	2
5·5	71	0·0114	0·0145	−0·0092	1
6·0	78	0·0138	0·0230	−0·0089	3

NOTE.—Q = −0·0082. Earliest intrinsic = −0·0138.

TABLE XXI.—Actual and Calculated Intrinsic Deflections.
(Left Ventricle, Dog G.V.)

Muscle thickness in mm.	Purkinje pathway in mm.	Calculated intrinsic.	Actual intrinsic.	Actual extrinsic.	Order of contact.
4·0	51	0·0082	0·0071	−0·0055	5
4·0	53	0·0086	0·0093	−0·0096	6
7·0	43	0·0126	0·0156	−0·0048	4
7·0	45	0·0130	0·0177	−0·0077	21
8·0	47	0·0154	0·0122	−0·0095	7
9·0	44	0·0168	0·0148	−0·0067	2
9·5	33	0·0156	0·0203	−0·0039	15
10·0	53	0·0206	0·0236	−0·0049	8
10·0	31	0·0162	0·0197	−0·0075	20
10·0	36	0·0172	0·0216	−0·0053	13
10·0	54	0·0208	0·0269	−0·0057	11
10·5	61	0·0232	0·0295	−0·0050	9
11·0	31	0·0182	0·0202	−0·0056	3
11·0	45	0·0210	0·0192	−0·0094	19
11·3	27	0·0180	0·0272	−0·0010	17
11·5	41	0·0212	0·0233	−0·0030	14
12·0	42	0·0224	0·0252	−0·0040	10
12·0	43	0·0226	0·0285	−0·0052	12
13·0	32	0·0224	0·0297	−0·0056	1

NOTE.—Q not measurable. Earliest intrinsic (internal) = −0·0100.

THE EXCITATION WAVE IN THE WHOLE HEART.

We are now in a position to summarise our observations upon the excitation wave in the heart as a whole.

That activity first reveals itself in the region of the sino-auricular node and in the neighbourhood of the head of that structure has been shown, we consider, beyond question. It spreads from this node in every direction, progressing to all the margins of the musculature; the propagation through the auricle may be likened to the spread of the fluid poured upon an almost flat surface. Distribution of the excitation wave throughout the auricular tissue as a whole is expedited by the central position at which it originates, by the convergence of the chief muscle band to this region and by a high rate of conduction in the auricular tissue.* Flowing through the auricle, the wave reaches the auriculo-ventricular node where it is delayed,† it spreads from this through the bundle and passes to the ventricle. In the muscle of the ventricle conduction is slowest, for its function of distribution is a minor one; rapidity of distribution is provided by the architecture of the special tissues. The main bundle splits into two branches, right and left, and then subdivides into a wide arborisation and communicates with a network which pervades almost the whole sub-endocardial space. This special system of fibres is endowed with the highest order of conducting power. The final spread through the muscle is by penetration of the whole thickness at innumerable points.

In both chambers of the heart a quick and rapid movement of the whole of the contractile tissue seems desirable. In the auricle, the smaller chamber, the plan is primitive, depending as it does upon the quality and arrangement of the contractile tissue itself; in the ventricle, the large and chief driving chamber, such a plan appears to be insufficient and so a system of distribution is specially provided, a system which has reached a high grade of development.

The system of distribution in the ventricle appears to us to possess a second virtue; it protects the tissues. If the contraction wave followed the separate muscle bands, these would contract separately and at different times. It is difficult to see how in such circumstances dissecting ruptures of the walls or laceration of the arterioles, which penetrate all layers at right angles, could be avoided.

It has been assumed tacitly in this report that conduction is through muscular and not nervous channels; this, the generally accepted view, is strongly supported by what appears to be a clear relation between conductive power and the constitution of the striated elements. Where glycogen is held most richly by the striated cells, conduction is fastest; where it is stored less richly, conduction is slower. There is a great delay in the region of the *A-V* node; the glycogen content ends abruptly as the bundle is traced into this node (NAGAYO, *loc. cit.*). Similarly, conduction is fastest where the striated cells are largest. Following the tissue elements from auricle to ventricle, a great decrease in size is observed at the *A-V* node, a sudden increase to the largest type of cell in the bundle and network, and a decrease at the junction of network and ventricular muscles. The variations of velocity are disposed in corresponding fashion.

---

* 'Phil. Trans.,' vol. 205, p. 375 (1914).

† LEWIS, WHITE and MEAKINS, 'Heart,' vol. 5, p. 289 (1913–14).

TABLE XXII.—A Table giving the Times and Values of the Initial Deflections of the Axial Electrocardiogram (Lead *II*) in those Animals from which Surface Readings are published in Illustrations.

Dog.	Fig.	Average heart rate.	*Q.*	*R.*	Summit of *R.*	Point of *S.*
G.K.	20	152	−0·0053 (0·5 mm.*)	0·0000	0·0155 and 0·0226 (11 and 16 mm.)	0·0423 (7·5 mm.)
G.L.	21	143	−0·0096	0·0000	0·0187 (12 mm.)	0·0424 (3 mm.)
G.M.	23	101	−0·0048	0·0000	0·0185 (23 mm.)	0·0376 (2 mm.)
G.T.	6	146	−0·0081 (1·0 mm.)	0·0000	0·0091 and 0·0269 (2 and 8 mm.)	0·0401 (3·5 mm.)
G.W.	7	106	−0·0107 (1·0 mm.)	0·0000	0·0259 (23 mm.)	None.
G.X.	8	152	−0·0082 (1·5 mm.)	0·0000	0·0228 (11 mm.)	0·0345 (1 mm.)
G.U.	9	161	−0·0105 (0·5 mm.)	0·0000	0·0221 (7 mm.)	None.
G.V.	10	176	None.	0·0000	0·0159 (6·5 mm.)	0·0407 (4 mm.)
G.Y.	11	195	−0·0066 (2·5 mm.)	0·0000	0·0193 (20 mm.)	None.

* 10 mm. = 1 millivolt. This table was added in proof stage (April 21, 1915).

EXPLANATION OF PLATE FIGURES.

Plate 8.

Fig. 19.—A projected drawing (twice natural size) of the interior of the right ventricle of a dog. The outer wall has been freed along the posterior interventricular groove and between the tricuspid and pulmonary valves and thrown downward. The ensheathed branches of the right division of the bundle are seen. The tracing is a drawing of the right branch of the bundle, the Purkinje arborisation and network, displayed by special carmine staining.

Fig. 20.—A projected drawing ($1\frac{2}{5}$ natural size) of the cavity of the right ventricle (Dog G.K.) showing the relations of the large papillary muscle and free arborisation to the trabeculated region and surface. The overlying tracings are projections of the superficial muscle fibres and of the surface of the heart with the contacts and readings of the experiment.

Fig. 21.—A similar projection ($1\frac{2}{5}$ natural size), with similar overlying tracings of the heart of Dog G.L.

Plate 9.

Fig. 22.—A projection (twice natural size) of the cavity of the left ventricle of a dog, opened between the two papillary muscles, and through the mitral valve. The tracing shows the left branch of the bundle, its arborisation and network. Prepared in similar fashion to fig. 19.

Plate 10.

Fig. 23.—A projection ($1\frac{2}{5}$ natural size) of the heart of Dog G.M., similar to that of fig. 20. The overlying tracings are projections of the cavity of the left ventricle, showing the relations of the papillary muscles to the surface, and of the surface of the heart with the contacts and readings of the experiment.

Plate 11.

Fig. 24.—A photograph ($\frac{3}{7}$ natural size) of the interior of the right heart of an ox, in which the sheaths of the Purkinje network have been injected. *p.v.* = pulmonary valves; *t.v.* = tricuspid valve segments: *c.b.* = moderator band. To show the extent of the network.

Fig. 25.—Dog G.J. Two curves ($\frac{2}{3}$ natural size), each simultaneous with th

standard electrocardiogram, showing the effects of changing a distal contact. The leads are illustrated in fig. 4 in the text.

Fig. 25 A.—The upper curve is from two contacts (*a–b*) on the left ventricle.

Fig. 25 B.—The upper curve is from point *a* on the left ventricle to the left chest wall (*c*). The form of curve in changing the distal contact from *b* to *c* alters considerably. The relation of the first extrinsic deflection to *R* (−0·0066 and −0·0100) varies; the relation of the intrinsic deflection to *R* (0·0188 and 0·0190) remains unchanged. *Ex.* = extrinsic; *In.* = intrinsic deflection.

Ordinates (upper curves), 5 scale divisions = 8·4 millivolts.
Abscissæ = 0·2 second.

Fig. 26.—Dog G.P. Four figures ($\frac{2}{3}$ natural size) showing the effect of cutting the chief branch of the right division of the bundle.

Figs. 26 A and 26 C are simultaneous records from a point on the right ventricle (upper curve in each) and from Lead *II* (lower curve in each). Figs. 26 B and 26 D are similar records from a point on the left ventricle Figs. A and B were taken before and figs. C and D after section of the branch of the bundle. The corresponding contacts may be found by consulting fig. 12.

Ordinates (upper curves), 5 scale divisions = 10 millivolts.
Abscissæ = 0·2 second.

Fig. 27.—Dog G.R. Four records ($\frac{2}{3}$ natural size) arranged in precisely the same fashion as those of fig. 26; and exemplifying a similar experiment. The corresponding contacts may be found by consulting fig. 13.

Ordinates (upper curves), 5 scale divisions = 10 millivolts.
Abscissæ = 0·2 second.

Plate 12.

Fig. 28.—Dog G.K. Two simultaneous curves (natural size) (1) from a conus to chest wall lead, and (2) from a point lower and more to the left on the conus.

As shown in the two cycles to the right of the figure, while the heart beat naturally, the excitation wave appeared almost simultaneously at the two conus points. While the heart responded to excitation at a point on the left ventricle in line with these contacts, the contact proximal to the point stimulated received the excitation wave 0·0119 second before the other (the distance of the contacts apart was 16 mm.).

Ordinates (both curves), 5 millimetres = 10 millivolts.
Abscissæ = 0·2 second.

Fig. 29.—Dog G.K. Similar curves from the same animal showing the slower conduction across the interventricular groove. (1) Curve taken from a lead from right ventricle to right chest wall. (2) Curve from a lead from left ventricle to left chest wall. The first three cycles were excited from the left border of the heart, at a point in line with the ventricular contacts; the latter lay 22 mm. apart, and the excitation wave took 0·0570 second to pass between them.

Ordinates (both curves), 5 millimetres = 10 millivolts.

Abscissæ = 0·2 second.

Fig. 30.—Dog G.O. Simultaneous curves (1) leading from an internal point near the base of the right ventricle to the right chest wall, (2) leading from an external point opposite the internal point to the left chest wall (see fig. 15).

While the heart beat naturally (last two cycles) the excitation wave at the internal point preceded that at the external point by an estimated time of 0·0205 second. While the heart beat in response to excitation at a point 15 mm. from the external contact, the excitation wave at the internal point preceded that at the external point by an estimated time of 0·0226 second.

Ordinates (both curves), 5 millimetres = 10 millivolts.

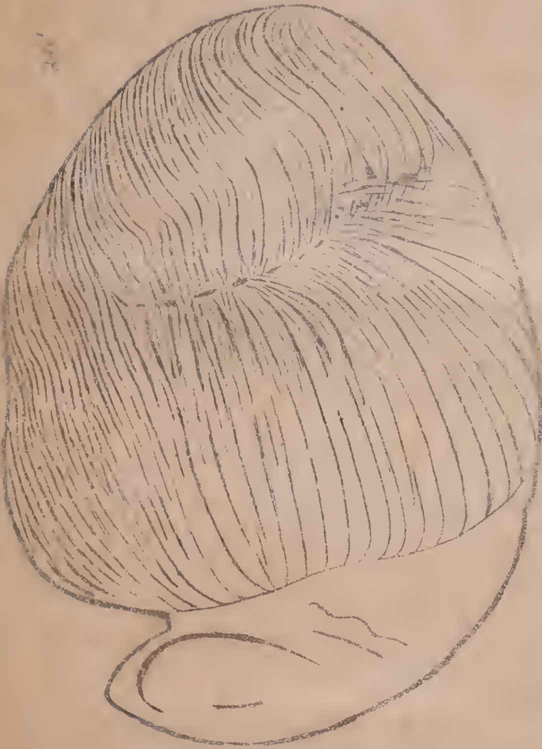
Abscissæ = 0·2 second.

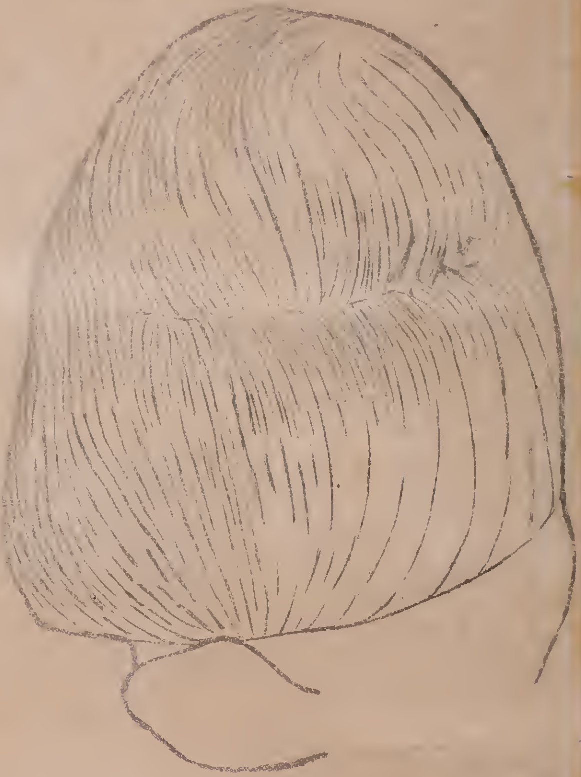
Fig. 31.—Dog G.O. An exactly similar experiment to that illustrated in fig. 30; with the exception that the point of stimulation lay only 6 mm. from the external point. The excitation wave at the internal point now precedes that at the external point by a lessened interval, namely, 0·0170 second, because the external point receives the excitation wave directly and not through the Purkinje system.

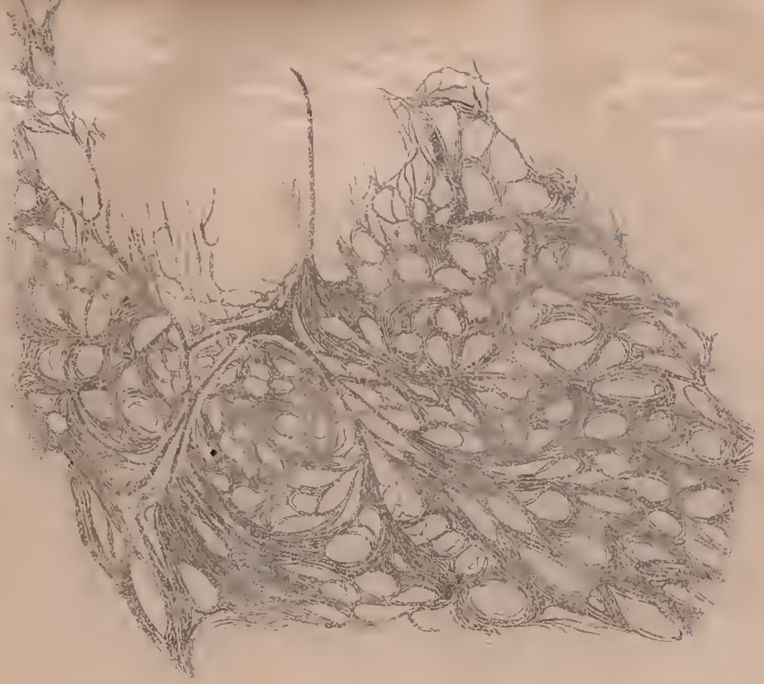
Ordinates (both curves), 5 millimetres = 10 millivolts.

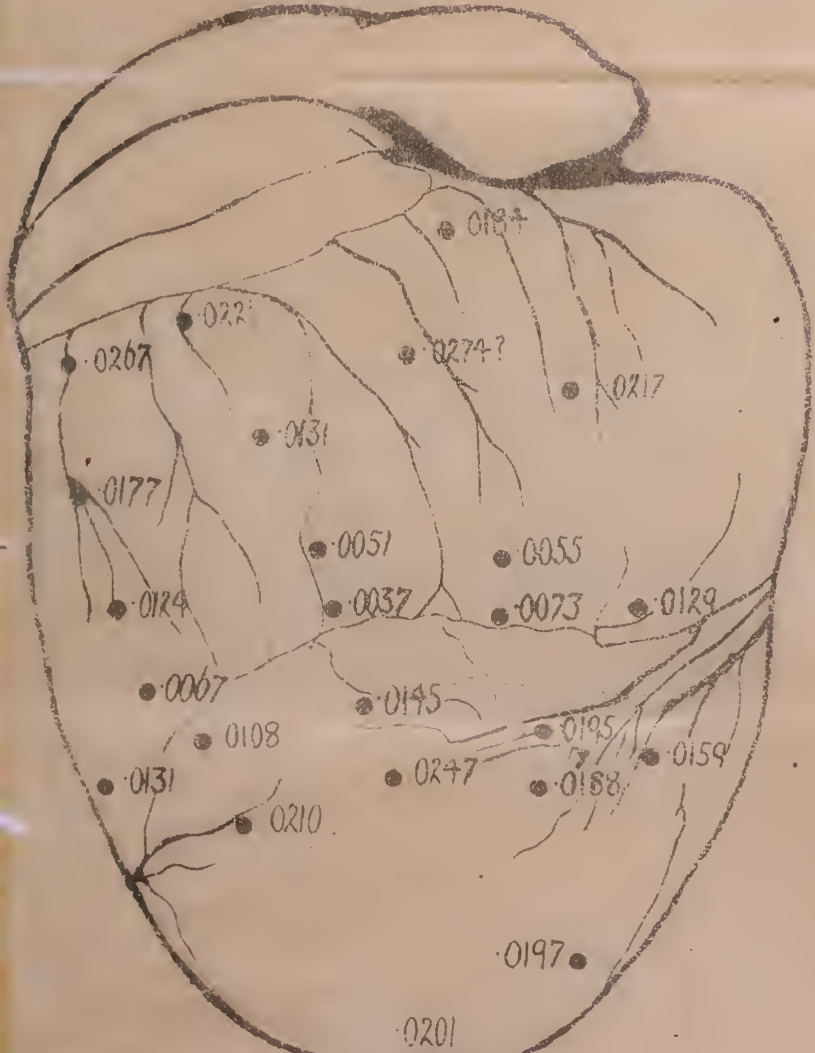
Abscissæ = 0·2 second.

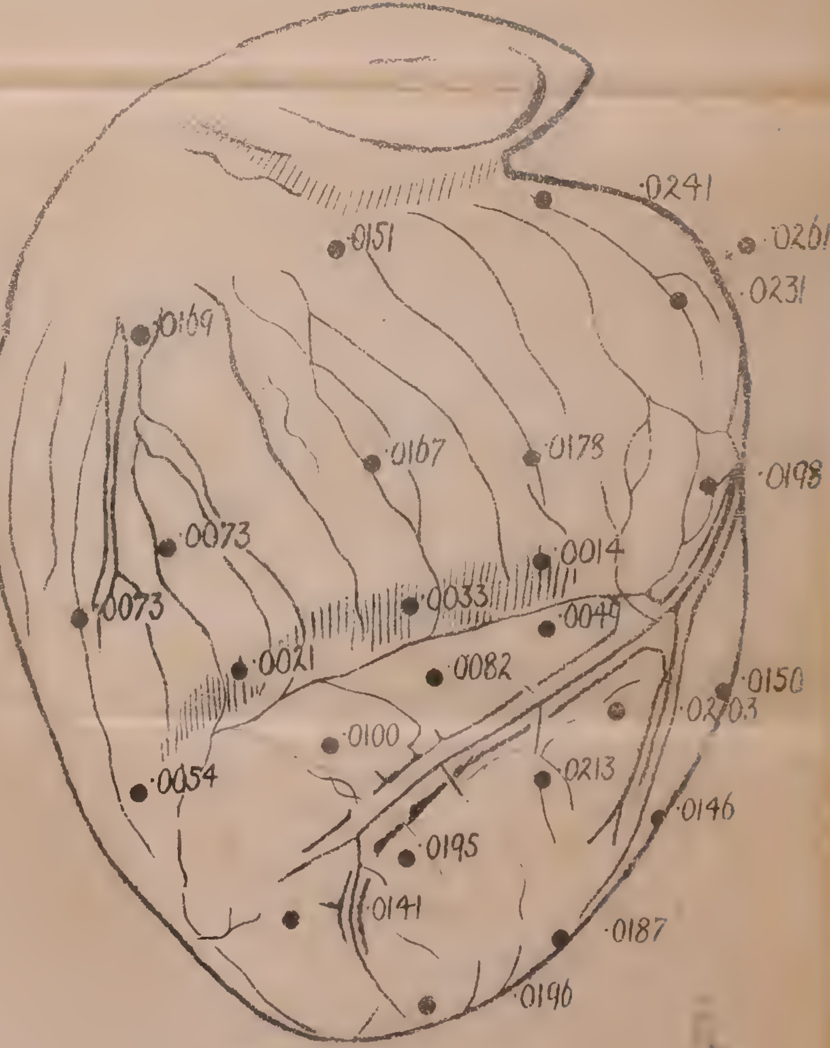
Note.—The ordinates for all standard curves (Lead *II*) are on the scale of 3 centimetres (30 scale divisions in reduced figures) = 3 millivolts.








·0184
·0221
·0267
·0274?
·0217
·0131
·0177
·0051
·0055
·0124
·0037
·0073
·0129
·0067
·0145
·0195
·0108
·0247
·0159
·0131
·0188
·0210
·0197
·0201


·0241
·0261
·0151
·0231
·0169
·0167
·0178
·0198
·0073
·0014
·0073
·0033
·0049
·0021
·0082
·0150
·0203
·0100
·0054
·0213
·0146
·0195
·0141
·0187
·0196

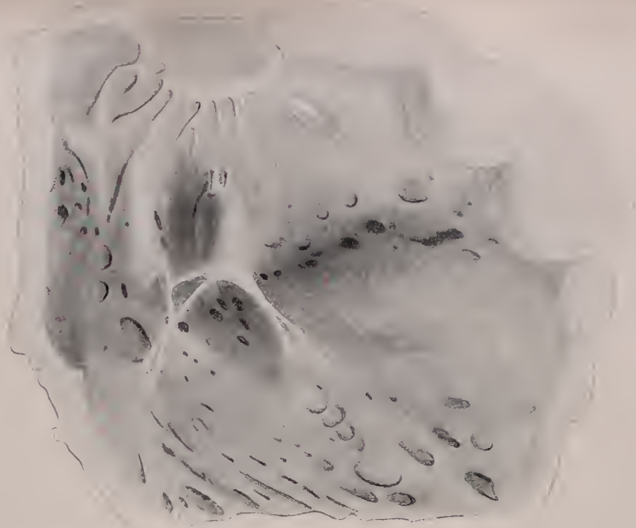


FIG. 19.

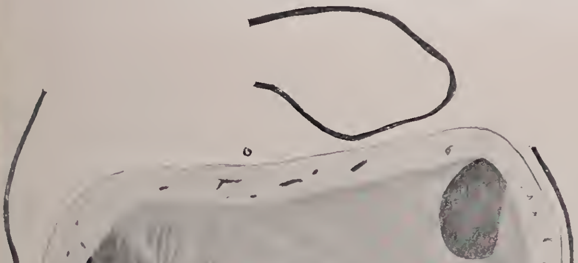


FIG. 20.

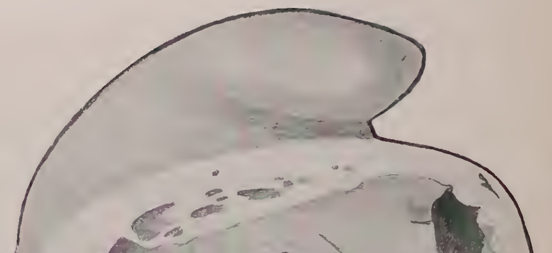


FIG. 21.

Article No. 7

a26 3

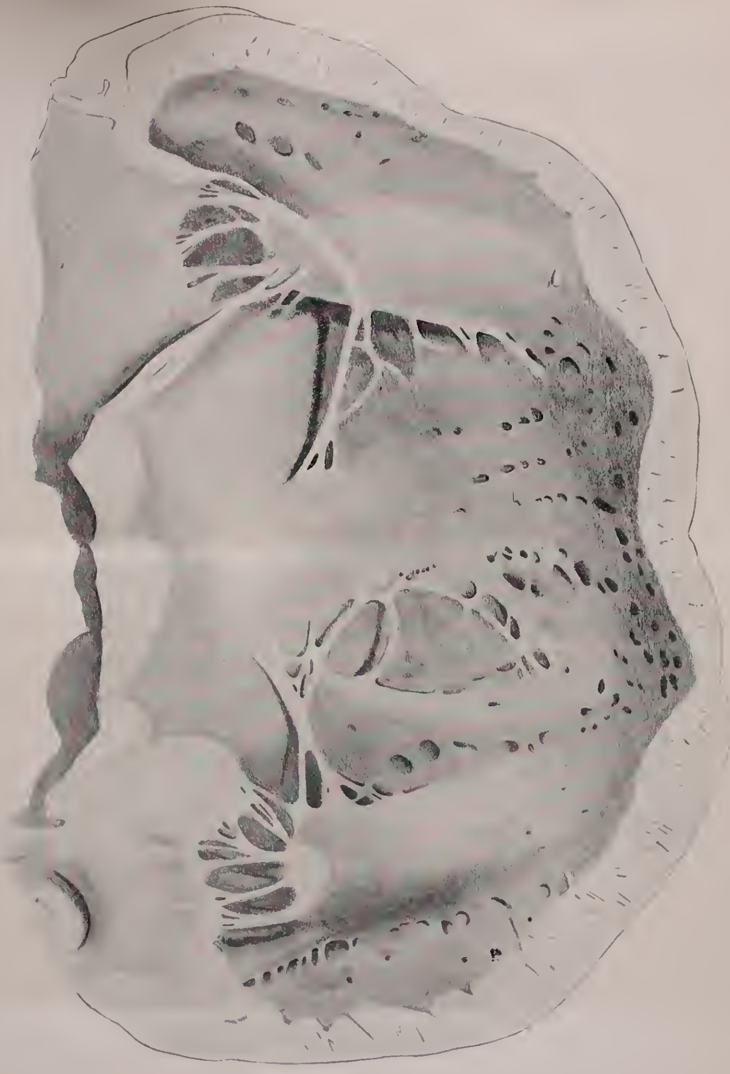
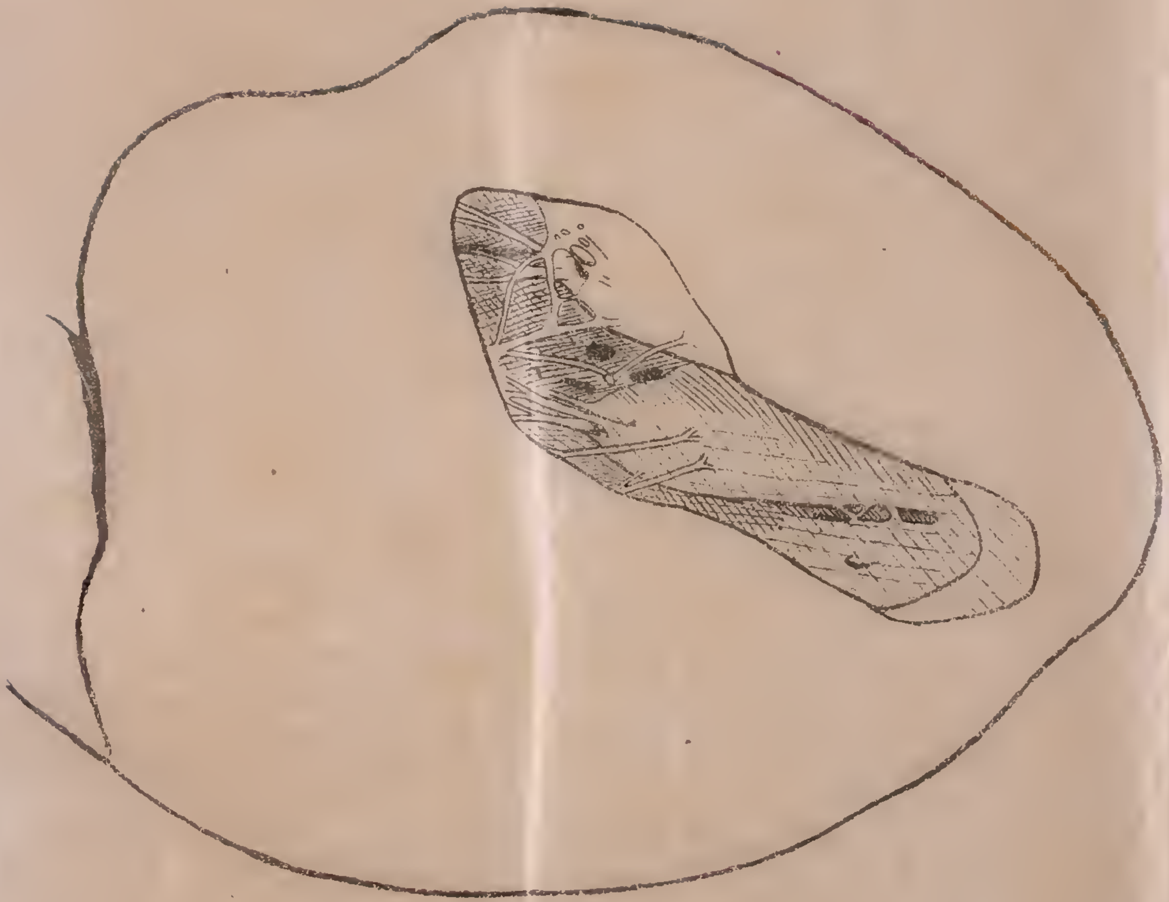
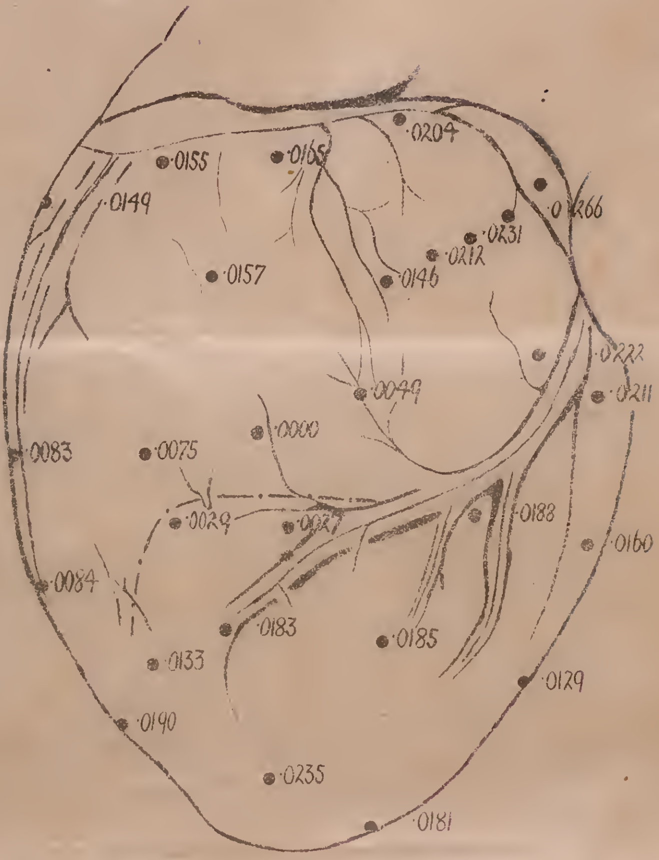


FIG. 22.

2260




·0204
·0155
·0165
·0149
·0266
·0231
·0212
·0146
·0157
·0222
·0049
·0211
·0000
·0083
·0075
·0029
·0027
·0188
·0160
·0084
·0183
·0185
·0133
·0129
·0190
·0235
·0181

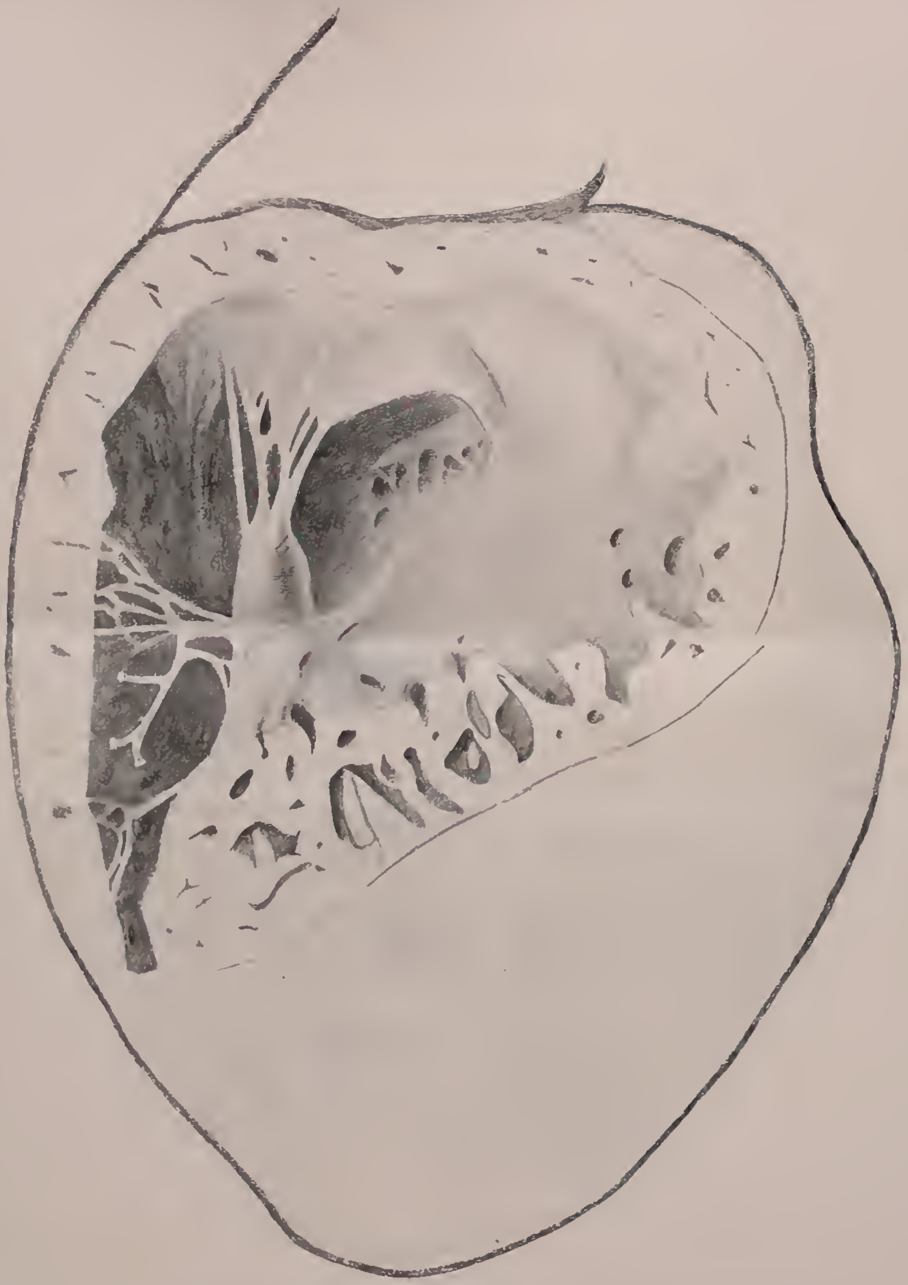


FIG. 23.

226

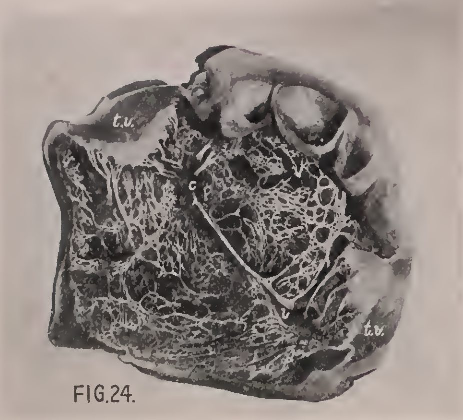
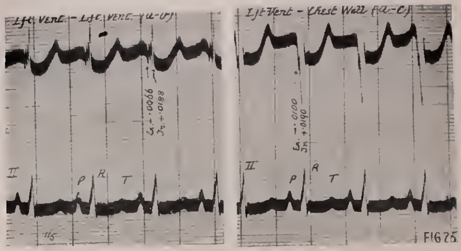
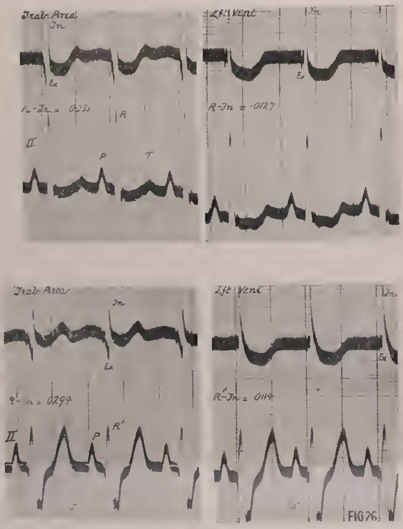


FIG. 24.



A. B.

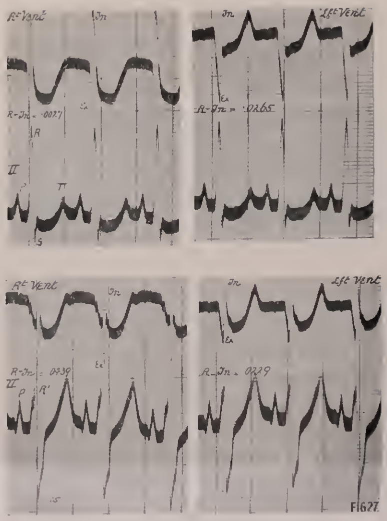
FIG. 25.



A. B.

C. D.

FIG. 26.



A. B.

C. D.

FIG. 27.

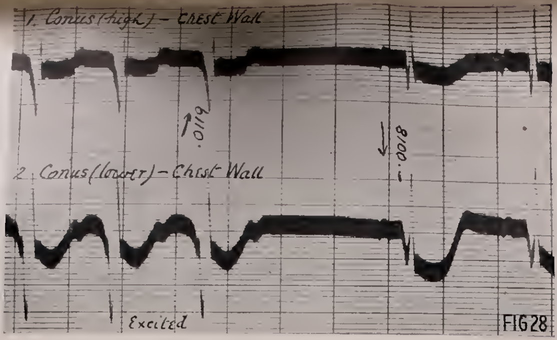


FIG. 28.

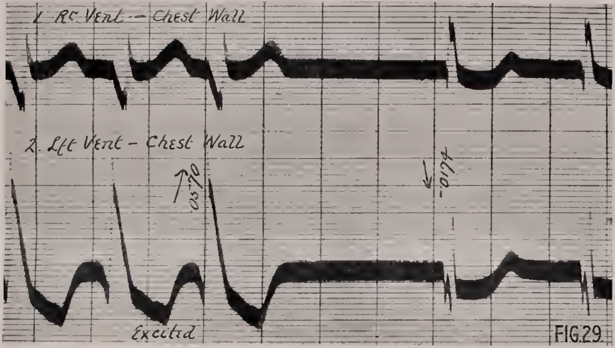


FIG. 29.

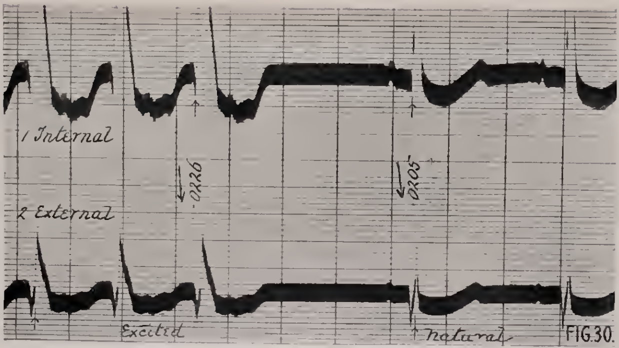


FIG. 30.

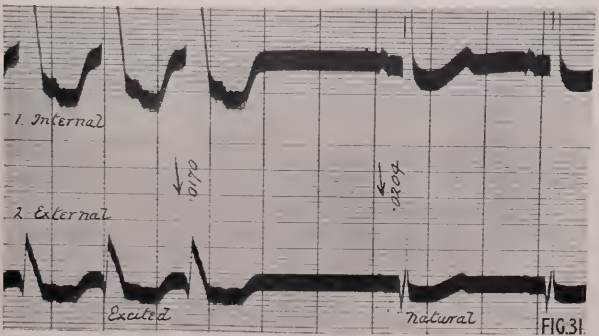


FIG. 31.

Journal of Pathology and Bacteriology, Vol. XIX.

No. 8

p. 349–371.

LIPOIDS IN IMMUNITY. (II.)[1]

WITH SPECIAL REFERENCE TO LIPOIDS OF THE TUBERCLE BACILLUS AND LIPOID ANAPHYLAXIS.

By F. H. Thiele and Dennis Embleton.

From the Bacteriological Laboratories, University College Hospital Medical School, London, W.C.

In a previous paper (1913 [1]),[2] we detailed our experiments showing that the lipoids such as those present in erythrocytes, tissue cells, and egg yolk, play no part in the production of the various known antibodies, such as lysins, agglutinins, precipitins, and the antibodies concerned in anaphylaxis. We showed that tissues, corpuscles, or other protein antigens, completely deprived of their lipoid constituents by repeated extractions with acetone, ether, and alcohol, produced on inoculation exactly the same variety and amount of antibody as is produced when the natural unextracted antigen is inoculated. Further, the inoculation of the lipoids themselves in a state of purity as regards protein contamination did not give rise to the formation of any of the above antibodies. Hence we concluded that the work of other observers showing the formation of antibodies, lysins, etc., by the inoculation of lipoids, and differences occurring when fat-free and unaltered antigen were inoculated, *e.g.*, absence of lysins and only the production of agglutinins with fat-free antigens, was erroneous and was due to the methods of extracting the antigen. Our work has been confirmed by Ritchie and Miller (1913 [2]) and Wilson (1914 [3]). Bogomolez (1910 [4]) also claimed that the removal of the lipoids from egg yolk so altered the antigen that the animal sensitised with the normal egg yolk did not react anaphylactically with fat-free egg yolk, and *vice versa*. We were unable to confirm his statements, and our observations (1913 [1]) have since been confirmed by Wilson (1913–14 [3]). From these experiments it may be concluded that the ordinary tissue lipoids do not act as antigens in the ordinarily accepted sense.

Certain observers have published experiments in which they claim to have demonstrated the presence of specific and non-specific lipases in the serum of

---

[1] [Received August 27, 1914.] Part of the expenses of this research was defrayed by a grant from the Government Grant Committee of the Royal Society of London.

[2] This paper is a continuation of the "Rôle of Lipoids in Immunity" (I.), in the *Ztschr. f. Immunitätsforschung*, 1913, Bd. xvi. S. 161.

animals inoculated with various lipoid-containing antigens. The work done on this subject is that of Neuberg (1908 [5]), who observed that the natural hæmolysins such as crotalus, cobra venom, etc., have a distinct lipolytic action on fats and lipoids like olive oil, castor oil, and lecithin. Neuberg and Reicher (1907 [6]) also found that antistreptococcic, bactericidal, and antitoxic sera had a similar property.

Again, Jobling and Bull (1913 [7]) showed that although there was not much difference between the non-specific lipase of normal and immune sera, as evidenced by their action on a foreign "fat" ethyl butyrate, there was some with the fat of the homologous antigen. In their estimations they carefully excluded acidity due to the formation of amido acids. They also drew a comparison between the *complementing* power of various sera and their lipolytic action on ethyl butyrate. These observers heated the normal and immune sera used to 56° C. for thirty minutes, and found that inactivation greatly reduced the lipolytic power. Neuberg and Reicher (1907 [6]) came to a similar conclusion. The peculiar point in all this work is the absolute want of correlation between the hæmolytic and lipolytic powers of the sera investigated. Jobling and Bull (1913 [7]) found that an inactivated serum when reactivated by complement, had only 50 per cent. of the original lipolytic power of the same serum (0·9 c.c. N/1 to 1·8 c.c. N/1 alkali per cent.); thus lipolysis and hæmolysis bear no relation to one another, and hæmolysis cannot be regarded as being due to a beginning lipolysis. Further (1913 [7]), in some experiments, after inactivation there was no lipolytic action at all on the addition of complement, but the lysis of the red cells was perfect.

In our previous experiments (1913 [1]), fat-free antigens produced the same amount and variety of antibodies as the ordinary non-extracted antigens. Hence hæmolysis cannot be due to beginning lipolysis, caused by the development of a specific lipolytic ferment to the corpuscular fats, etc. Fats, when they get into the body, remain like the original fats, as they were till they are taken to the liver and desaturated and converted into lecithins (Kennaway and Leathes, 1909 [8]), and there is no reason why a lipase should be formed against them, as the body is, normally, perfectly capable of dealing with foreign fats. It has been shown that normal serum has no true lipolytic action (Siebert, 1909 [9]; Thiele, 1913 [10]), and hydrolysis of ethyl butyrate cannot be taken as evidence that the serum contains a true lipase.

In this paper we propose to detail our experiments with regard to

I.—The formation of lipases on inoculation of phosphatids and fats and the occurrence of lipases in hæmolytic sera.

II. (*A*).—The lipoids of the tubercle bacillus, and (*B*) the formation of antibodies against the various lipoids of the tubercle bacillus, as evidenced by

(*a*) Lipolytic power.
(*b*) Precipitin formation.
(*c*) Complement fixation.
(*d*) Anaphylaxis.

I. In the experiments here quoted, the "specific" and "non-specific" lipolytic power of various sera was tested under absolutely aseptic conditions; all experiments showing evidence of bacterial contamination were discarded.

1. Hæmolytic Sera.

The serum of a rabbit highly immune against sheep's red cells was removed aseptically. This serum was tested fresh and after inactivation against six times carefully washed sheep's red cells, which had been washed with sterile saline in sterile tubes. The complement was also obtained aseptically. The units of the

(1) active serum,
(2) inactivated serum,
(3) complement,

were obtained, and mixtures made with the red cells and active serum, or inactivated serum and complement in quantities, so that hæmolysis should be complete in thirty minutes at 36° C. The mixtures were incubated for two,

EXPERIMENT 1.—Two Hours' Incubation.

Acidity in c.c. of alkali N/100.

No.		Ether Extract.	Alcohol Extract.	Total.
1	25 c.c. red cells (control)	6·5	7·5	14·0
2	25 ,, ,, (incubated)	6·75	8·1	14·8
3	25 ,, ,, + hæmolytic serum . . .	7·2	8·0	15·2
4	25 ,, ,, + ,, ,, (incubated)	7·2	10·3	17·5
5	25 ,, ,, + inactivated hæmolytic serum + complement (control)	7·4	8·1	15·5
6	25 ,, ,, + inactivated hæmolytic serum + complement (incubated)	7·5	10·4	17·9

EXPERIMENT 2.—Four Hours' Incubation.

1	25 c.c. red cells (control)	6·2	6·8	13·0
2	25 ,, ,, (incubated)	7·5	9·2	16·7
3	25 ,, ,, + hæmolytic serum (control) .	6·4	7·0	13·0
4	25 ,, ,, + ,, ,, (incubated) .	7·6	11·8	19·4
5	25 ,, ,, + inactivated hæmolytic serum + complement (control)	6·8	7·9	14·7
6	25 ,, ,, + inactivated hæmolytic serum + complement (incubated)	8·0	12·3	20·3

EXPERIMENT 3.—Eight Hours' Incubation.

1	25 c.c. red cells (control)	6·0	6·6	12·6
2	25 ,, ,, (incubated)	8·3	12·4	20·7
3	25 ,, ,, + hæmolytic serum (control) .	6·8	6·8	13·4
4	25 ,, ,, + ,, ,, (incubated) .	8·9	15·7	24·6
5	25 ,, ,, + heated hæmolytic serum + complement (control)	7·2	7·4	14·6
6	25 ,, ,, + heated hæmolytic serum + complement (incubated)	9·4	16·0	25·4

EXPERIMENT 4.—Twelve Hours' Incubation.

1	25 c.c. red cells (control)	6·2	7·0	13·2
2	25 ,, ,, (incubated)	9·6	14·2	23·8
3	25 ,, ,, + hæmolytic serum (control) .	7·2	7·8	15·1
4	25 ,, ,, + ,, ,, (incubated) .	10·2	16·8	27·0
5	25 ,, ,, + heated hæmolytic serum + complement (control)	7·2	8·0	15·2
6	25 ,, ,, + heated hæmolytic serum + complement (incubated)	10·3	16·9	27·2

four, six, eight, twelve hours. Controls were always put up. After incubation the mixtures were poured into shallow dishes and rapidly dried *in vacuo.* The material was then finely powdered and boiled with a measured quantity of absolute alcohol for six hours in a flask with a reflux condenser. After this the mixture was evaporated to dryness and extracted in a Soxhlet with ether for twenty-four hours. All the extractions were made with measured quantities of alcohol and ether, and the same amounts were used throughout, so that the same error should occur all through with regard to any acid contained in the alcohol and ether. After the ether extraction the residue was boiled up with absolute alcohol again. The ethereal and alcoholic extracts thus obtained were titrated with N/100 alcoholic potash.

For these experiments on analysis see Table I.

TABLE I.

		Autolytic Lipolysis.	Autolytic Lipolysis + Lipolysis from active Serum.	Autolytic Lipolysis + Lipolysis from inactivated Sera.
Experiment 1 .	2 hours	0·25	0·0	0·1
,, 2 .	4 ,,	1·3	1·2	1·2
,, 3 .	8 ,,	2·3	2·1	2·2
,, 4 .	12 ,,	3·4	3·0	3·1

It will be seen that the serum has no lipolytic action at all, but in fact, probably because of its reaction, inhibits the autolytic lipolytic ferment. The ethereal acidity is thus due to the fatty acids split off from the phosphatids by the autolytic lipolytic ferment of the red cells themselves (Thiele, 1914[10]), so that there is no relation between hæmolysis and lipolysis. This is confirmatory of the results obtained by the inoculation of "fat-free" antigens and lipoids. If no note is taken of the above, then a slight lipolytic action would be manifest. Now 1 c.c. N/100 alkali = 0·00282 grm. of oleic acid, so that even then the amount hydrolysed is very small. If, however, the total acidity is taken as meaning fatty acids, then of course there is apparently a marked "lipolytic" action, but if the amido-acids are estimated separately then the error is manifest.

2. To test the lipolytic action on a foreign phosphatid.

A thick suspension of purified ox heart lecithin was made in normal saline and sterilised at 60° C. for one hour.

[EXPERIMENTS 1–3

EXPERIMENT 1.—INCUBATION SIX HOURS.

All made up with normal saline to 5 c.c. Then as before. Acidity of ether extract, N/200 alkali c.c.

	Control.	Incubated.
1 c.c. lecithin	10·0	10·2
1 c.c. lecithin + 1 c.c. complement	8·5	8·0
1 c.c. lecithin + 1 c.c. fresh hæmolytic serum	10·0	10·0
1 c.c. lecithin + 1 c.c. of heated hæmolytic serum + 1 c.c. of complement	7·5	8·0

From this it will be seen that in six hours no hydrolysis of the lecithin had taken place.

EXPERIMENT 2.—INCUBATION TWENTY-FOUR HOURS.

Method like Experiment 1, N/200 alkali.

1 c.c. of lecithin	10·0	10·0
1 c.c. of lecithin + 1 c.c. complement	9·2	9·3
1 c.c. of lecithin + 1 c.c. fresh hæmolytic serum	9·6	9·6
1 c.c. of lecithin + 1 c.c. heated hæmolytic serum + 1 c.c. complement	8·8	8·7

EXPERIMENT 3.—INCUBATION FORTY-EIGHT HOURS.

N/200 alkali.

1 c.c. lecithin	10·0	10·4
1 c.c. + 1 c.c. complement	9·2	9·4
1 c.c. + 1 c.c. fresh hæmolytic serum	9·6	9·6
1 c.c. + 1 c.c. heated hæmolytic serum + 1 c.c. complement	8·8	9·0

Thus showing completely negative results, *i.e.*, there is no true lipase either specific or non-specific in a hæmolytic serum.

3. Inoculation of the phosphatids of sheep's red cells into guinea-pigs.

These were chosen instead of rabbits because the amount of phosphatid obtainable from large quantities of blood was very small. The guinea-pigs were given five injections of 100 mgrms. each of the phosphatid, intraperitoneally, at intervals of two days. The animals were kept alive for three weeks and then killed. The serum was found in none of them to have developed any hæmolytic power to sheep's red corpuscles.

To test the lipolytic power, a 1 per cent. suspension of the phosphatid was made in normal saline and sterilised. 5 c.c. were mixed with 2 c.c. of the serum and tested in the same way as above.

EXPERIMENT 1.	Acidity N/100 alkali c.c.
5 c.c. lecithin suspension + 2 c.c. serum (control)	1·2
5 ,, ,, + 2 ,, (incubated 4 hours) . .	1·4
5 ,, ,, + 2 ,, (,, 12 hours) . .	1·2
5 ,, ,, + 2 ,, (,, 24 hours) . .	1·8
EXPERIMENT 2.	
5 c.c. lecithin suspension + 2 c.c. serum (control)	1·4
5 ,, ,, + 2 ,, (incubated 4 hours) . .	1·2
5 ,, ,, + 2 ,, (,, 8 hours) . .	1·4
5 ,, ,, + 2 ,, (,, 24 hours) . .	1·8
EXPERIMENT 3.	
Same result.	

Hence the injection of a tissue phosphatid does not produce a hæmolysin or specific lipolytic ferment.

4. Lipolytic action of immune sera.

TABLE II.

ANTISTREPTOCOCCIC SERUM.—Incubated 48 hours.	Control.	Incubated.
5 c.c. lecithin suspension + 3 c.c. antiserum	7·0	7·2
5 ,, ,, + 3 ,, ,, + 1 c.c. complement	6·8	6·7
5 ,, ,, + 1 ,, complement	6·4	6·4

All made up to 10 c.c. and treated as above.

Other experiments give similar results, namely, that immune sera do not contain a true lipase.

Eckstein (1913 [11]) has brought forward some experiments to show that syphilitic sera giving a marked Wassermann reaction have after inactivation a lipolytic action on fats, whereas the fresh sera have no such action. He used sesame oil for his experiments. We (1914 [12]) have already shown that the antigenic power of the phosphatids in the Wassermann reaction is not due to the nature or variety of the phosphatids used, but depends on the presence of an "active" substance obtained from the tissues by autolytic degradation before extraction, and brought out with the phosphatid during the process of extraction. Since we have shown that the phosphatid removed from a perfectly fresh organ was not "antigenic," we thought it would be interesting to note whether syphilitic sera had a lipolytic action on these phosphatids. We accordingly tried the action of such sera on the ox heart phosphatid, but found that such a lipolytic ferment did not exist (see Table III.).

TABLE III.

Method as before.

Syphilitic Serum.	Acidity N/200 Alkali.	
	Control.	Incubation.
1 c.c. lecithin suspension in normal saline + 2 c.c. fresh serum. Incubation 24 hours	5·4	6·0
1 c.c. lecithin suspension in normal saline + 2 c.c. fresh serum. Incubation 48 hours	5·4	6·0
1 c.c. lecithin suspension in normal saline + 2 c.c. serum heated. Incubation 24 hours	5·2	6·0
1 c.c. lecithin suspension in normal saline + 2 c.c. serum heated. Incubation 48 hours	5·4	6·0

Other experiments gave similar results, the differences being all in the limit of experimental error. We therefore conclude that syphilitic sera have no lipolytic action on phosphatids, and the antigenic value of the latter is not due to complement fixation occurring caused by such a lipolytic ferment. From these results we conclude—

1. There is no relationship between the hæmolytic and lipolytic power of immune sera.
2. That hæmolysis is not due to a beginning lipolysis.
3. Inoculation of phosphatids does not produce either a hæmolysin or a specific lipase.

II. (*A*).—THE LIPOIDS OF THE TUBERCLE BACILLUS.

A very large quantity of dry tubercle bacilli was obtained from

Meister, Lucius Brüning & Co. This was extracted by the following succession of solvents :—

1. By repeated extractions with acetone at 37° C. till no more extractives were removed.
2. Then by extracting with ether in a Soxhlet for forty-eight hours.
3. Subsequently extracting repeatedly with absolute alcohol at 37° C. till no more extraction took place.
4. To remove any phosphatid that had not been removed, extraction was again carried out with ether in the Soxhlet for twenty-four hours.
5. The residue was then treated with trichlor-ethylene at 37° C. to remove the wax.
6. The residue was next treated with dilute NaOH in the water bath for some hours, neutralised, and evaporated to dryness. The residue was then extracted with ether.

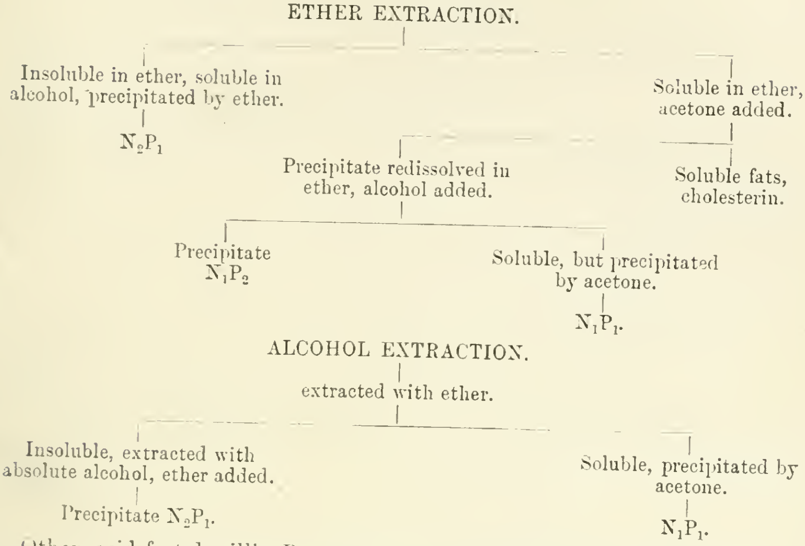
By this method of extraction the acetone removed the neutral fats, cholesterin, fatty acids, and colouring matter. The ether subsequently removed the phosphatids which are not closely bound to the protein. The greater part of the phosphatid obtained in this way was insoluble in acetone and alcohol, and corresponds to the N_1P_2, the cuorin fraction of Erlandsen (1907 [13]) and others. On drying it formed a yellowish-white brittle substance and was not auto-oxidisable. It was readily redissolved in ether. In addition there was a comparatively small amount of phosphatid soluble in alcohol and ether, and insoluble in acetone. It is much more hygroscopic than the N_1P_2 fraction and also somewhat auto-oxidisable, and had the properties of the N_1P_1 fraction of a tissue phosphatid.

The subsequent alcohol-ether extraction (step 3, *supra*) removed only small amounts of phosphatid. This was in part N_1P_1 phosphatid, soluble in ether and alcohol, insoluble in acetone, and having the characters of the similar fraction of the primary ether extract. In addition, there was a fair amount of extract which would not go into ethereal solution, but was precipitated by ether from the alcoholic solution. It thus corresponded to the N_2P_1 fraction. A small amount of a similar fraction was present in the primary ether extract.

The trichlor-ethylene (step 5, *supra*) removed a waxy substance which on drying became yellowish, very tenacious, and transparent. It was soluble in alcohol and ether. The bacilli even after treatment up to this stage had not lost their acid-fastness.

The treatment with dilute soda (step 6, *supra*) extracted a white crystalline substance, soluble in ether, corresponding to part of the "Nastin" of Deycke and Much. This substance melted on heating. After its removal the bacteria were no longer acid-fast.

The lipoids thus obtainable from the tubercle bacilli are—


ETHER EXTRACTION.
Insoluble in ether, soluble in alcohol, precipitated by ether.
N_2P_1
Soluble in ether, acetone added.
Precipitate redissolved in ether, alcohol added.
Soluble fats, cholesterin.
Precipitate N_1P_2
Soluble, but precipitated by acetone.
N_1P_1.
ALCOHOL EXTRACTION.
extracted with ether.
Insoluble, extracted with absolute alcohol, ether added.
Precipitate N_2P_1.
Soluble, precipitated by acetone.
N_1P_1.

Other acid-fast bacilli, *B. smegma*, *B. rabinoritch*, were extracted in a similar way with similar qualitative results.

II. (*B*).—ANTIBODIES PRODUCED BY THE INOCULATION OF THE TUBERCLE LIPOIDS, ETC.

In these experiments guinea-pigs and rabbits were immunised by repeated intra-peritoneal inoculations of either

(*a*) Pulverised tubercle bacilli; (*β*) extracted tubercle bacilli; (*γ*) tubercle phosphatid.

Guinea-pigs received three to five successive doses of 20 mgrms. of the various varieties of tubercle bacilli, or three successive doses of 20 mgrms. of the tubercle phosphatids. Rabbits received five doses in the same way. The animals were bled ten days after the last inoculation, the sera were inactivated at 56° C. for half an hour and tested—

(*a*) Un-inactivated for lipolytic power; (*b*) for precipitin; (*c*) for complement fixation; (*d*) for sensitising properties.

(A) EXPERIMENTS DEALING WITH THE LIPOLYTIC POWER OF IMMUNE SERA AGAINST THE TUBERCLE PHOSPHATID.

EXPERIMENT 1.

Mixtures were made with 100 mgrms. of tubercle phosphatid and 5 c.c. of the immune sera. Methods as above.

	Acidity of Ether Extract, N/200 Alkali.
Control unincubated	8·2 c.c.
After 2 hours' incubation	8·0 ,,
,, 8 hours' ,, ...	8·4 ,,
,, 24 hours' ,, ...	8·8 ,,

Other experiments gave similar results. Hence the lipolytic power of the tubercle phosphatid (immune serum) is very slight, if present at all, against its antigen.

(B) PRECIPITINS.

The following tables (IV.–V.) give the results of the experiments dealing with the precipitin reaction of the sera of guinea-pigs and rabbits immunised

with (*a*) Tubercle phosphatids, N_1P_2.
(*β*) Whole tubercle bacilli.
(*γ*) Extracted tubercle bacilli.
with (*a*) Tubercle phosphatids. }
(*b*) Whole tubercle bacilli. } as antigens.
(*c*) Extracted tubercle bacilli. }

The phosphatid used gave absolutely no protein reactions, the ninhydrin test was perfectly negative.

PRECIPITINS AND AGGLUTININS.

I. PHOSPHATID SERA AGAINST PHOSPHATID SUSPENSIONS.

TABLE IV.—*Dilutions of Phosphatid Serum N_1P_2.*

	Antigen N_1P_2.	I. Guinea-pig.			II. Guinea-pig.			III. Rabbit.		
		1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.
Phosphatid Emulsion in normal saline.	Dilution—									
	1 in 10 . .	+++++	+++++	++++	+++++	++++	++	++++	+++	+
	1 in 100 . .	+++++	++++	++	+++++	+++	+	+++	+	0
	1 in 1000 . .	+++++	+++	0	++++	+	0	+	0	0

TABLE V.—*Dilutions of Phosphatid Serum N_1P_2.*

Antigen N_1P_1.	I. Guinea-pig.			II. Guinea-pig.			III. Rabbit.		
	1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.
Dilution—									
1 in 10 . . .	+++++	+++++	++++	+++++	+++	0	++++	+++	0
1 in 100 . . .	+++++	+++	+	++++	++	0	+++	+	0
1 in 1000 . . .	++++	+++	0	+++	+	0	0	0	0

From which it will be seen that the "phosphatid" serum has a marked precipitating power against both the N_1P_2 and N_1P_1 fractions. The precipitating power is practically the same against N_1P_1 fraction as against the homologous antigen.

Table VI.

II. WHOLE AND EXTRACTED TUBERCLE SERA AGAINST THE CORRESPONDING ANTIGENS AND THE PHOSPHATIDS.

Antigen.	PULVERISED TUBERCLE SERUM.					
	Guinea-pig.			Guinea-pig.		
	1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.
	Dilution of Sera.			Dilution of Sera.		
Pulverised tubercle bacilli—						
1 in 10 . . .	+++++	++++	+++	+++++	+++++	++++
1 in 100 . . .	++++	++	+	++++	+++	++
1 in 1000 . . .	+++	++	0	+++	++	+
Extracted tubercle bacilli—						
1 in 10 . . .	+++++	++++	+++	+++++	++++	+++
1 in 100 . . .	++++	+++	+	+++++	+++	++
1 in 1000 . . .	+++	+	+	++	+	0
Phosphatid N_1P_2—	1 in 10.	1 in 50.	1 in 100	1 in 10.	1 in 50.	1 in 100.
1 in 10 . . .	+++++	+++++	++++	+++++	+++++	++++
1 in 100 . . .	++++	++++	++++	++++	++++	++++
1 in 1000 . . .	++++	+++	+	++++	+++	++
Phosphatid N_1P_1—	1 in 10.	1 in 50.	1 in 100	1 in 10.	1 in 50.	1 in 100.
1 in 10 . . .	+++++	+++++	++++	+++++	+++++	+++++
1 in 100 . . .	++++	++	++	++++	+++	+++
1 in 1000 . . .	+++	++	+	++++	+++	++

Antigen.	EXTRACTED TUBERCLE BACILLI SERUM.					
	Guinea-pig.			Guinea-fig.		
	1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.
	Dilution of Sera.			Dilution of Sera.		
Pulverised tubercle bacilli—						
1 in 10 . . .	+++++	+++++	+++	++++	+++	+
1 in 100 . . .	++++	++++	+++	+++	+	+
1 in 1000 . . .	++++	+++	+	++	+	+
Extracted tubercle bacilli—						
1 in 10 . . .	+++++	++++	++	++++	++++	+++
1 in 100 . . .	++++	+++	+	+++	++	+
1 in 1000 . . .	+++	++	0	+	+	+
Phosphatid N_1P_2—	1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.
1 in 10 . . .	+++++	+++++	++++	+++++	++++	++++
1 in 100 . . .	++++	++++	+++	+++	++	+
1 in 1000 . . .	+++	++	+	+	+	+
Phosphatid N_1P_1—	1 in 10.	1 in 50.	1 in 100.	1 in 10.	1 in 50.	1 in 100.
1 in 10 . . .	+++++	+++++	++++	+++++	++++	++++
1 in 100 . . .	++++	+++	+++	++++	+++	+
1 in 1000 . . .	++	+	0	+	+	0

These experiments show that :—

1. The whole tubercle bacilli not only provoke precipitins against the whole bacillus, but also against the fat-free bacilli. The precipitin is not quite so active against the fat-free as against the whole bacilli.
2. The fat-free bacilli produce precipitins against the whole bacilli as well as against fat-free bacilli.
3. The whole bacilli produce a powerful precipitin against the tubercle phosphatids.
4. The fat-free bacilli do the same, but it is not so powerful as that produced by the whole bacilli.
5. Animals immunised against tubercle phosphatid develop a precipitin against the phosphatid. This is in marked contrast to the results we previously (1913 [1]) obtained on injecting animals with tissue phosphatid.

(C) COMPLEMENT FIXATION REACTION.

In these experiments the sera of animals inoculated with

(*a*) whole tubercle bacilli,
(*β*) extracted tubercle bacilli,
(*γ*) tubercle phosphatid,
(*δ*) "Nastin,"

were tested against all the various antigens. Table VII. gives the complete results of a series of experiments thus carried out.

These experiments show that—

1. Immune sera produced against whole tubercle bacilli have complement fixating antibodies which react with
    (*a*) whole bacilli,
    (*b*) extracted bacilli,
    (*c*) phosphatid,
    (*d*) "Nastin" as antigens,

    and there is not much difference between the fixating powers with different antigens.

2 "Extracted bacilli" anti-sera react

    (1) well with $\begin{cases}(a) \text{ whole bacilli,}\\(b) \text{ extracted bacilli,}\end{cases}$
    (2) very slightly with "Nastin,"
    (3) with the phosphatids, but much less powerfully than with the bacillary proteins. The reaction is also less marked with these sera with the phosphatid antigen than with the whole bacilli sera and phosphatid antigen.

TABLE VII.

Antigen.		Guinea-pig in c.mm.			Guinea-pig in c.mm.			Rabbit in c.mm.		
Description.	Quantity.	100.	10.	1.	100.	10.	1.	100.	10.	1.
ANTISERA *VERSUS* PULVERISED TUBERCLE BACILLI.										
Pulverised tubercle bacilli.	100 c.mm. = 1 in 1000.	+++++	++++	++	+++++	+++++	+++	++++	+++	+
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	+++++	+++	+	+++++	++++	+	++++	+	0
		Strong.			Very strong.			Marked.		
Extracted tubercle bacilli after trichlor-ethylene.	100 c.mm. = 1 in 1000.	+++++	+++++	+++	+++++	+++++	+++	+++++	+++	++
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	+++++	+++	+	+++++	+++++	+	+++++	++	+
		Strong.			Very strong.			Strong.		
Extracted tubercle bacilli after NaOH.	100 c.mm. = 1 in 1000.	+++++	+++++	+++	+++++	+++++	++++	+++++	+++++	+++
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	+++++	+++	+	+++++	+++	+	+++++	+++++	+
		Strong.			Very strong.			Strong.		
Phosphatid cuorin N_1P_2.	100 c.mm. = 1 in 1000.	+++++	+++++	+++	+++++	+++++	+++	+++++	+++++	++++
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	+++++	+++	++	+++++	++++	++	+++++	++	0
		Very strong.			Very strong.			Strong.		
"Nastin."	100 c.mm. = 1 in 1000.	+++++	+++	+	+++++	+++++	+++	+++++	++++	+++
0·1 grm. in 10 c.c. absolute alcohol.	10 c.mm. = 1 in 10,000.	++++	+	0	+++++	+++	+	+++++	++	+
		Strong.			Very strong.			Strong.		
ANTISERA *VERSUS* EXTRACTED TUBERCLE BACILLI AFTER TRICHLOR-ETHYLENE.										
Pulverised tubercle bacilli.	100 c.mm. = 1 in 1000.	++++	+++	+	+++	+++	+	+++++	++++	++
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	++++	+	0	+++	+	0	+++++	+++	+
		Marked.			Marked.			Strong.		
Extracted tubercle bacilli after trichlor-ethylene.	100 c.mm. = 1 in 1000.	+++++	+++	+++	+++++	+++	+	+++++	+++++	+++
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	+++++	+++	+	+++++	+	0	+++++	+++	+
		Strong.			Strong.			Very strong.		
Extracted tubercle bacilli after NaOH.	100 c.mm. = 1 in 1000.	+++++	++++	++	+++++	+++	+	+++++	+++++	+++
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	+++++	+++	+	++++	+	0	+++++	++	+
		Strong.			Strong.			Strong.		
Phosphatid cuorin N_1P_2.	100 c.mm. = 1 in 1000.	++++	+++	+	++	+	0	++++	+++	+
0·1 grm. in 10 c.c. saline.	10 c.mm. = 1 in 10,000.	+++	+	0	+	0	0	++++	+	0
		Marked.			Slight.			Marked.		
"Nastin."	100 c.mm. = 1 in 1000.	++	++	0	++	+	0	+++	++	0
0·1 grm. in 10 c.c. absolute alcohol.	10 c.mm. = 1 in 10,000.	+	0	0	+	0	0	+	+	0
		Slight.			Slight.			Slight.		

TABLE VII.—*continued.*

ANTISERA *VERSUS* EXTRACTED TUBERCLE BACILLI (AFTER NaOH.)

ANTIGEN.		GUINEA-PIG IN C.MM.			GUINEA-PIG IN C.MM.			RABBIT IN C.MM.		
Description.	Quantity.	100.	10.	1.	100.	10.	1.	100.	10.	1.
Pulverised tubercle bacilli. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ + +	+ + +	+	+ + + +	+ + +	+	+ + + + +	+ + +	+ +
	10 c.mm. = 1 in 10,000.	+ + +	+	0	+ + +	+	0	+ + + + +	+ +	+
		Marked.			Marked.			Strong.		
Extracted tubercle bacilli, after trichlorethylene. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ + + + +	+ + +	+	+ + + + +	+ + +	+	+ + + + +	+ + + + +	+ + +
	10 c.mm. = 1 in 10,000.	+ + + +	+	0	+ + + +	+	0	+ + + + +	+ + +	+
		Marked.			Strong.			Very strong.		
Extracted tubercle bacilli, after NaOH. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ + + +	+ + +	+	+ + +	+ + +	+	+ + + + +	+ + +	+
	10 c.mm. = 1 in 10,000.	+ + +	+	0	+ + +	+	0	+ + + +	+	0
		Marked.			Marked.			Strong.		
Phosphatid cuorin N_1P_2. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ +	+ +	0	+ + +	+ + +	+	+ + + +	+ + +	+
	10 c.mm. = 1 in 10,000.	+	+	0	+ + +	+	0	+ +	+	0
		Slight.			Marked.			Marked.		
"Nastin." 0·1 grm. in 10 c.c. absolute alcohol.	100 c.mm. = 1 in 1000.	+ + +	+ +	0	+ +	+ +	+	+ + + +	+ + +	+
	10 c.mm. = 1 in 10,000.	+	0	0	+	0	0	+ + +	+	0
		Slight.			Slight.			Marked.		
ANTISERA *VERSUS* PHOSPHATID N_1P_2.										
Pulverised tubercle bacilli. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ + +	+ +	+	+ + +	+ + +	+	+ + + + +	+ + + + +	+ + +
	10 c.mm. = 1 in 10,000.	+ + +	+	0	+ + +	+	0	+ + + + +	+ +	+
		Marked.			Marked.			Strong.		
Extracted tubercle bacilli, after trichlorethylene. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ + + + +	+ + +	+	+ + + + +	+ + + + +	+ + +	+ + + + +	+ + + + +	+ + + +
	10 c.mm. = 1 in 10,000.	+ + + +	+	0	+ + + + +	+ +	+	+ + + + +	+ + + +	+
		Strong.			Strong.			Very strong.		
Extracted tubercle bacilli, after NaOH. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ + + + +	+ + + + +	+ +	+ + + + +	+ + + + +	+ + +	+ + + + +	+ + + + +	+ + +
	10 c.mm. = 1 in 10,000.	+ + + + +	+	0	+ + + + +	+ +	+	+ + + + +	+ + + +	+
		Strong.			Strong.			Very strong.		
Phosphatid cuorin N_1P_2. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ + + + +	+ + + + +	+ + + +	+ + + + +	+ + + + +	+ + +	+ + + + +	+ + + + +	+ + + +
	10 c.mm. = 1 in 10,000.	+ + + + +	+	0	+ + + + +	+ + +	+	+ + + + +	+ + + +	+
		Strong.			Very strong.			Very strong.		
"Nastin." 0·1 grm. in 10 c.c. absolute alcohol.	100 c.mm. = 1 in 1000.	+ + + +	+ +	+	+ + + +	+ + +	+	+ + + +	+ + +	+
	10 c.mm. = 1 in 10,000.	+ + +	+	0	+ + + +	+	0	+ + +	+	0
		Marked.			Marked.			Marked.		

Table VII.—*continued.*

ANTISERA *VERSUS* "NASTIN."							
Antigen.		Guinea-pig in c.mm.			Guinea-pig in c.mm.		
Description.	Quantity.	100.	10.	1.	100.	10.	1.
Pulverised tubercle bacilli. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ +	0	0	+ +	+	0
	10 c.mm. = 1 in 10,000.	+	0	0	+	0	0
		Very slight.			Slight.		
Extracted tubercle bacilli, after trichlor-ethylene. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ +	+ +	0	+ +	0	0
	10 c.mm. = 1 in 10,000.	+	0	0	+	0	0
		Slight.			Very slight.		
Extracted tubercle bacilli, after NaOH. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ +	+ +	0	+ +	+ +	+
	10 c.mm. = 1 in 10,000.	+	0	0	+	0	0
		Slight.			Slight.		
Phosphatid cuorin N_1P_2. 0·1 grm. in 10 c.c. saline.	100 c.mm. = 1 in 1000.	+ +	+	0	+ + +	+ + +	+
	10 c.mm. = 1 in 10,000.	+	0	0	+ + +	+	0
		Slight.			Marked.		
"Nastin." 0·1 grm. in 10 c.c. absolute alcohol.	100 c.mm. = 1 in 1000.	+ + + +	+ + +	+	+ + +	+ +	+
	10 c.mm. = 1 in 10,000.	+ + + +	+	0	+ + +	+	0
		Marked.			Marked.		

3. Phosphatid sera react
    (1) well with whole bacilli and extracted bacilli,
    (2) vigorously with the phosphatids,
    (3) less markedly with "Nastin."
4. "Nastin" immune sera practically only react with "Nastin," very slightly with the other antigens.

(D) ANAPHLYLACTIC EXPERIMENTS.

ACTIVE SENSITISATION.

Series I.—Guinea-pigs were inoculated, 18th March 1914, intraperitoneally with 20 mgrms. of the alcohol insoluble phosphatid (N_1P_2) of the tubercle bacillus, obtained from a fresh quantity of dry tubercle bacilli. This phosphatid when tested gave no reaction with ninhydrin, showing the absence of any α-amino containing constituents or protein.

April 27, 1914.

Guinea-pig 1 (280 grms.).—Received intravenously 20 mgrms. of the same phosphatid, carefully ground up in 2 c.c. of saline. On injection, rapidity of breathing, convulsions, respiratory spasm, death in two minutes.

Post-mortem.—The lungs were pale, markedly emphysematous. The right side of the heart was distended, the heart continued pulsating for some time. The blood clotted readily.

GUINEA-PIG 2 (310 grms.).—20 mgrms. tubercle phosphatid intravenously. Death in five minutes after completion of inoculation, convulsions, respiratory spasms, cyanosis.

Post-mortem.—The lungs were only slightly distended, and there was commencing intravascular clotting.

GUINEA-PIG 3 (360 grms.).—50 mgrms. tubercle phosphatid intravenously. Death in two minutes after completion of inoculation. Same symptoms as No. 2.

Post-mortem.—Appearance same, together with some intravascular clotting.

GUINEA-PIG 4 (280 grms.).—Inoculated intravenously, 5 mgrms. B.E. tuberculin. No result, beyond what occurs in normal animal with 5 mgrms. B.E. intravenously.

GUINEA-PIG 5 (324 grms.).—Inoculated intravenously, 7·5 mgrms. B.E. tuberculin. Some delay in recovery, rapidity of breathing, weakness but little more than could be accounted for by the tuberculin itself. Complete recovery in twenty minutes.

GUINEA-PIG 6 (320 grms.).—Inoculated intravenously, 10 mgrm. B.E. tuberculin. Respiratory quickening, paresis, twitching, delayed recovery. Quite well in one hour.

Controls.

GUINEA-PIG A (280 grms.).—20 mgrms. of the tubercle phosphatid intravenously. Animal quite well.

GUINEA-PIG B (318 grms.).—100 mgrms. of the tubercle phosphatid intravenously. No symptoms.

It will thus be seen that

1. The tubercle phosphatid even in large doses did not produce any symptoms, thrombosis, or death in an unsensitised guinea-pig when inoculated intravenously.
2. Guinea-pigs, sensitised five weeks previously with the tubercle phosphatid, reacted when re-inoculated with the antigen, the symptoms and mode of death being similar to those occurring with the typical anaphylaxis to protein antigens, the only deviation being in the rapid coagulation of the blood in these experiments.
3. Guinea-pigs thus sensitised did not react with the bacillary emulsion, *i.e.*, the "whole" bacillary constituents.

Series II.—Guinea-pigs inoculated with 20 mgrms. of the alcohol insoluble phosphatid of the tubercle bacillus, 4th June 1914. The phosphatid did not give any protein reactions.

June 26, 1914.

GUINEA-PIG 1 (312 grms.).—100 mgrms. tubercle phosphatid intravenously. Respiratory spasm, convulsions, death in one minute.

Post-mortem.—Typical lung condition. No intravascular clotting.

GUINEA-PIG 2 (286 grms.).—20 mgrms. tubercle phosphatid intravenously. Rapid breathing, Cheyne Stokes; twitchings, convulsions, cessation of breathing, death in seven minutes.

Post-mortem.—As in guinea-pig 1. No thrombosis.

Guinea-pig 3 (295 grms.).—20 mgrms. tubercle phosphatid intravenously. Same as guinea-pig 2.

Post-mortem.—Some intravascular clotting, otherwise as above.

Guinea-pig 4 (304 grms.).—10 mgrms. tubercle phosphatid intravenously. Rapid breathing, paresis, urination, etc., delayed recovery.

Guinea-pig 5 (316 grms.).—100 mgrms. of N_1P_1 phosphatid from ox heart intravenously. No effect.

Guinea-pig 6 (306 grms.).—2·5 mgrms. B.E. tuberculin intravenously. No apparent effect.

Guinea-pig 7 (290 grms.).—5 mgrms. B.E. tuberculin intravenously. Very ill, cessation of breathing for a time, started again. Convulsion jerks, rigors, paresis. Gradual recovery.

Guinea-pig 8 (320 grms.).—7·5 mgrms. B.E. tuberculin intravenously. Same as guinea-pig 7. Recovery.

Guinea-pig 9 (314 grms.).—20 mgrms. tubercle phosphatid intraperitoneally. Animal quite well for thirty minutes, then rapidity of breathing, rigors, urination, fall of temperature from 103°·0 F. to 96° F.

Controls.

Guinea-pig A (282 grms.).—100 mgrms. of tubercle phosphatid inoculated intravenously. Animal absolutely unaffected.

Guinea-pig B (300 grms.).—100 mgrms. of tubercle phosphatid inoculated intravenously. Animal absolutely unaffected.

These results of the experiments in this series are very similar to those of the previous series, except that the intravascular clotting was not so marked. They show further that there is a slight hypersensitiveness on the part of the sensitised animals to the whole bacilli, as exhibited in the form of bacillary emulsion. There is no hypersensitiveness to heterologous phosphatid.

Series III.—To test the sensitiveness to the tubercle phosphatid of guinea-pigs inoculated with

(*a*) pulverised tubercle bacilli,
(*b*) pulverised fat-free tubercle bacilli,

the two series of pigs were inoculated with these antigens on 4th June 1914.

June 26, 1914.—**Series III.** (*a*).

Guinea-pig 1.—20 mgrms. tubercle phosphatid intravenously. Some rapidity of breathing, weakness, rapid recovery.

Guinea-pig 2.—40 mgrms. tubercle phosphatid intravenously. Same as guinea-pig 1.

Guinea-pig 3.—100 mgrms. tubercle phosphatid intravenously. Very ill, cessation of breathing, restarted spasmodically, ceased again, then slow respiratory recovery, paralysis.

Guinea-pig 4.—Same dose. Same result.

Series III. (*b*).

Guinea-pig 1.—100 mgrms. tubercle phosphatid intravenously. Exactly the same as guinea-pigs 3 and 4 of Series III. (*a*).

Guinea-pig 2.—40 mgrms. tubercle phosphatid intravenously. No apparent result.

Guinea-pig 3.—5 mgrms. B.E. tuberculin intravenously. Acute typical death.

The results of Series III. (*a*) and III. (*b*) show that animals, sensitised to the whole bacilli and fat-free bacilli, reacted somewhat to the tubercle lipoid.

PASSIVE SENSITISATION.

Previous experience (1913 [14]) had demonstrated to us that passive sensitisation only succeeds if the proper amount of sensitising antibody is inoculated. For a hæmolytic serum we have shown that for a given system the hæmolytic serum will only passively sensitise if a definite number of amboceptor units are present in the amount of serum inoculated. With hypersensitive guinea-pigs the titre is so low that passive sensitisation with the sera of such animals can only succeed if huge quantities of serum, pooled from several animals, are used. If, however, the serum is derived from animals that have had repeated injections, the antibody titre is greatly increased, and consequently the amount necessary for passive sensitisation is greatly diminished.

We found exactly the same with the serum of guinea-pigs actively hypersensitive to the tubercle phosphatid. Taking animals that had only received a single injection of 20 mgrms. of the phosphatid, we were unable to passively sensitise guinea-pigs of 250 to 300 grms. with doses up to 15 c.c. of the mixed sera from such hypersensitive animals, and since we found that all the other members of the series were actively hypersensitive, it follows that the failure of the serum to sensitise was not due to the serum being derived from animals which were not hypersensitive, but that the antibody titre was too low.

In order to test this the serum was taken from six guinea-pigs which had been immunised by four injections of 20 mgrms. each of the tubercle phosphatid intraperitoneally; this was injected in varying

Table VIII.

	Sensitising Serum.	Provocative Dose.	Result.
Guinea-pig 1	5 c.c.	100 mgrms. phosphatid intravenously.	Nil.
,, 2	10 ,,	100 mgrms. phosphatid intravenously.	Paresis, rigors, sneezing fits, recovery.
,, 3	15 ,,	40 mgrms. phosphatid intravenously.	Prolonged coma, Cheyne Stokes, paresis, fall of temperature, slow recovery.
,, 4	15 ,,	40 mgrms. phosphatid, previously sensitised with immune serum.	Prolonged coma, Cheyne Stokes, paresis, fall of temperature, but death in two hours.
,, 5	15 ,,	100 mgrms. phosphatid intravenously.	Prolonged coma, paresis, Cheyne Stokes, fall of temperature, rigors, recovery.

quantities intraperitoneally into a series of guinea-pigs of 250 to 300 grms. Forty-eight hours later 40 to 100 mgrms. of the phosphatid were inoculated intravenously (see Table VIII.).

It thus appears that passive sensitisation of normal guinea-pigs can occur with blood of homologous immunised animals, though acute death did not occur. This may be due to the weakness of the sensitised serum, or to the peculiarity of the guinea-pigs, since certain batches of animals do not react so acutely as others, and the batch from which the above came was such a one. These experiments therefore show that—

1. *Guinea-pigs can become sensitised with protein-free tubercle phosphatids.*
2. *Guinea-pigs thus sensitised react with tubercle phosphatid, and only slightly with an emulsion of the whole tubercle bacilli (B.E.).*
3. *The symptoms produced by the re-injection are very similar to those produced in protein anaphylaxis, but differ in the relatively frequent occurrence of intravascular thrombosis.*
4. *Guinea-pigs sensitised with whole tubercle bacilli react with the tubercle phosphatid, but not so vigorously as the phosphatid sensitised ones.*
5. *Passive sensitisation can occur.*

FORMATION OF "ANAPHYLOTOXIN" *IN VITRO.*

The serum of some immune guinea-pigs was taken and mixed with tubercle phosphatid (N_1P_2) in the proportion of 100 mgrms. per 5 c.c. The mixture was thoroughly shaken and incubated at 37° C. and the serum tested at intervals.

TABLE IX.

After 1 hour . .	5 c.c. intravenously	No result.
,, 2 hours . .	,, ,,	The animal became weak, paresed, had rigors, but soon recovered.
,, 3 ,, . .	,, ,,	Same result as above.
,, 4 ,, . .	,, ,,	As above, but recovery much more prolonged.
,, 8 ,, . .	,, ,,	No result.

We were thus unable to produce an acutely toxic substance by incubating the phosphatid with the immune serum.

These results are interesting and important for the following reasons :—

1. It is the first time that true phosphatid hypersensitiveness has been demonstrated. It differs from that demon-

strated by K. Meyer (1914 [15]) for the tape-worm lipoid hypersensitiveness in that, whereas he was able only to produce anaphylactic symptoms by re-injecting with the tape-worm protein, we obtained the symptoms corresponding to protein anaphylaxis by re-injecting the phosphatid, and could not get a fatal result by re-injecting with the bacillary protein (B.E.) in doses not acutely toxic by themselves (1913 [16]).

2. The certainty of producing this hypersensitiveness. K. Meyer (1914 [15]) was able only to obtain it with tape-worm lipoid in 50 per cent. of his experiments; we obtained it in every one.
3. K. Meyer (1914 [15]) was unable to obtain symptoms by reinoculating with the tape-worm phosphatid, guinea-pigs sensitised with the tape-worm protein. Guinea-pigs sensitised with tubercle bacilli protein, however, react with tubercle phosphatid, but not as vigorously as do phosphatid sensitised ones.

Thus true hypersensitiveness to the tubercle phosphatid can be brought about. The questions, however, of paramount importance are—

A. Is the reaction due to the liberation of some cleavage body from the phosphatid capable of producing the symptoms of anaphylaxis, such as convulsions, paresis, respiratory spasm, etc.?

B. Is the reaction due to an alteration in the colloidal state in the animals' tissues, produced by the interaction of the antigen and antibody?

C. Is the death due to a thromboplastic phase, the result of the interaction of the antigen and antibody?

There can be no doubt but that the phosphatid we used was free from protein contamination, since

(*a*) it did not give the ninhydrin test,

(*b*) animals sensitised with it reacted only slightly with the whole tubercle protein.

Hence if the antibody reacting were really one against the tubercle protein, the sensitising dose was so small as to fail to give a ninhydrin reaction, and the firing off dose of the phosphatid contained similarly such small amounts as to fail to give this test. Now, although it has been shown that very minute quantities of protein can sensitise, yet for re-inoculation appreciably larger quantities have to be used. When whole bacilli were used for re-inoculation, the reaction was only moderate; hence it cannot be argued that the above-quoted results, namely, acute death on re-injection with the phosphatid, were due to the formation of an antibody against the tubercle protein from protein contamination of the phosphatid.

The reaction also cannot be due to a lipolytic action of the hypersensitive serum on the phosphatid, since we have already shown, and it has been confirmed by other observers, that animals inoculated with pure phosphatids of animal origin do not react on re-injection of such phosphatids, and we have shown (*vide supra*) that there is no true lipolytic ferment in such sera, or in the case of the serum of animals immunised against the tubercle phosphatid. The only possibility is that the nucleus of the tubercle phosphatid may have a peculiar composition, complex enough in nature to provoke antibody formation after the type of that produced against proteins; and as the result of the interaction of this antibody with its antigen, substances may be liberated from the phosphatid nucleus capable of producing the symptoms noted.

With regard to *B*, there seems to be no doubt but that acute anaphylactic shock is produced by the interaction of antigen and antibody locally in the tissues; and if we believe with Dale (1913 [20]) that the death in the guinea-pig is due to asphyxia associated with bronchial spasm—following alterations in surface tension produced by the interaction of the antigen and antibody—this acute lipoid anaphylaxis may be well explained on this ground, namely, death due to bronchial spasm produced by the interaction of the anti-lipoid antibody and the antigen. Since we have demonstrated the presence of a precipitin to the phosphatid, the reaction is quite parallel to that occurring in egg-albumin sensitised animals, and if the explanation holds for the latter, it, similarly, can be held for the tubercle phosphatid anaphylaxis.

With regard to *C*, we have the feature that the clotting of the blood of the sensitised animals on reinjection is very rapid, that intravascular clotting has been noted, and in two cases (Series I., guinea-pigs 2 and 3) the animals apparently died from intravascular clotting, the lungs not being distended. Death is very much like that produced by the injection of coagulation-producing substances, *e.g.*, colloidal silicic acid, iron salts, etc., as has been shown by ourselves (1914 [17]), and later by Kretschmer (1914 [18]). But too much stress must not be laid on this, since in some experiments carried out by us on double sensitisation, when the animals had been temporarily desensitised to one antigen, the subsequent injection of the other antigen produced acute anaphylaxis, and the outstanding feature noted was the rapid clotting of the blood, quite different from that usually seen in anaphylactic shock.

The outstanding feature in these experiments is that animals inoculated with tubercle phosphatid or tubercle protein produced antibodies—precipitins, complement fixating and anaphylactic, which reacted best with the specific antigen, but which also reacted with the other. Now, K. Meyer (1913 [19]) in his work on the tubercle lipoids, endeavoured to make out a complete specificity for the antibodies produced to the two varieties of antigen by complement fixation tests, but when he came to the question of specific adsorption, he found that this did not hold, but that the protein removed not only the antibody to the protein, but also that against the lipoid. Similarly, although he was unable to produce acute anaphylaxis by the injection of the tape-worm phosphatid into the phosphatid sensitised animals, he was able to produce anaphylaxis by reinjecting with the tape-worm protein, hence the anaphylactic antibody against tape-worm lipoid reacted in the animal body only with tape-worm protein—a further example of the want of specificity of the antibodies—against the protein and phosphatid of the tape-worm. This is quite in agreement with our own observations in these experiments, where the antibodies present in a given serum reacted best with the antigen against which they were provoked, but also did so with the other portions of, or with the whole, antigen from which the sensitising antigen was derived, not only *in vitro* but *in vivo*. Meyer believes that there may be, in addition to the antibodies against the lipoid and protein, one present against lipo-protein. Now there is no evidence on this point. Bogomolez (1910 [4]) originally brought forward experiments trying to prove this, but subsequent work by ourselves (1913 [1]) and others (Ritchie and Miller, 1913 [2], and Wilson, 1913–14 [3]) has shown that animals sensitised with lipo-protein (egg-yolk) react equally well with the unaltered or the fat-free antigen, and *vice versa* in the case of animals sensitised with fat-free protein. This is further negatived by K. Meyer's own experiments on the tape-worm lipoid, where the antibody to the lipoid reacts with the lipo-protein or protein, which it should not if the antibodies produced against the two antigens are different. Hence, it would appear that the antibodies provoked by the tubercle bacillary protein and phosphatid are not absolutely specific for the antigen used in provoking them.

SUMMARY.

1. The hæmolytic power of a serum bears no relationship to its lipolytic power. A hæmolysin is not a lipase.

2. Antibodies cannot be provoked against the ordinary tissue fats lipoids or phosphatids.

3. The phosphatids of the tubercle bacillus can, however, provoke antibody formation.

Those produced are—

Precipitins.
Bordet Gengou.
Anaphylactic antibodies.

These are not strictly specific, but react not only with the specific antigen, but with the whole or lipoid-free antigen from which the phosphatid was obtained.

4. Guinea-pigs can be rendered actively hypersensitive to the phosphatids of the tubercle bacillus by a previous inoculation with those phosphatids.

Such animals are also somewhat hypersensitive to the proteins of the tubercle bacillus, and *vice versa* guinea-pigs sensitised with tubercle protein react mildly with the bacillary phosphatid.

5. The symptoms and mode of death occurring on the reinoculation of such hypersensitive animals are identical with those occurring in acute protein anaphylaxis.

REFERENCES.

1. Thiele and Embleton . *Ztschr. f. Immunitäts-forsch.*, (Orig.) 1913, Bd. xvi. S. 161.
2. Ritchie and Miller . *Journ. Path. and Bacteriol.*, Cambridge, 1913, vol. xvii. p. 429.
3. Wilson . *Ibid.*, 1913–14, vol. xviii. p. 163.
4. Bogomolez . *Ztschr. f. Immunitäts-forsch.*, (Orig.) 1910, Teil 1, Bd. v. S. 121; 1910, Bd. vi. S. 332.
5. Neuberg . *Biochem. Ztschr.*, 1908, Bd. xi. S. 400.
6. Neuberg and Reicher . *Ibid.*, 1907, Bd. iv. S. 287.
7. Jobling and Bull . *Journ. Exper. Med.*, New York, 1913, vol. xvii. p. 61.
8. Kennaway and Leathes . *Lancet*, London, 1909, vol. i. p. 95.
9. Siebert . *Ztschr. f. physiol. Chem.*, Strassburg, 1900, Bd. xlix. S. 50.
10. Thiele . *Biochem. Journ.*, 1913, vol. vii. p. 287.
11. Eckstein . *Ztschr. f. Immunitäts-forsch.*, 1913, vol. xviii. p. 107.
12. Thiele and Embleton . *Lancet*, London, 1914, vol. i. p. 526.
13. Erlandsen . *Ztschr. f. physiol. Chem.*, Strassburg, 1907, Bd. li. S. 71.
14. Thiele and Embleton . *Ztschr. f. Immunitäts-forsch.*, (Orig.) 1913, Teil. 1, Bd. xx. S. 159.
15. Kurt Meyer . *Ibid.*, (Orig.) 1914, Teil 1, Bd. xxi. S. 654.
16. Thiele and Embleton . *Ibid.*, 1913, Bd. xvi. S. 411.
17. „ „ . *Ibid.*, 1914, Bd. xx. S. 159.
18. Kretschmer . *Biochem. Ztschr.*, 1914, Bd. lviii. p. 399.
19. K. Meyer . *Ztschr. f. Immunitätsforsch.*, (Orig.) 1913, Teil 1, Bd. xix. S. 313.
20. Dale . *Journ. Pharmacol. and Exper. Ther.*, 1913, vol. iv. p. 167.

No 9

pp 372 — 391

Journal of Pathology and Bacteriology, Vol. XIX.

THE MECHANISM OF ANTIBODY ACTION.[1]

By F. H. Thiele and Dennis Embleton.

From the Bacteriological Laboratory, University College Hospital Medical School, London.

As the result of the work of Ehrlich and his school the conception has become firmly rooted that complement does not behave like an enzyme but works quantitatively and forms a direct firm combination with the antigen it attacks. The reason for this is that complement action has always been studied from the point of view of ascertaining the minimal dose which will do a certain amount of work in a standard time, and in order to do more work in that time it has been found that an increased amount is necessary. This gave rise to the idea of the quantitative action of complement and the misconception of its real mode of action.

Now, when a universally recognised enzyme is studied under like conditions, the same relationships will be found to hold. Thus dealing with the proteolytic enzyme trypsin, it will be found that, taking the liquefaction of gelatin as a gauge of its activity, in order to do a definite amount of work in a standard time a definite quantity of the ferment is necessary. If now the amount of work to be done in the same time be increased, it will be found that the amount of trypsin necessary to do the work will be greater. Under these conditions an enzyme, like a complement, may be stated to work quantitatively. It is only when no note is taken of the time factor that the ferment can be said to work according to the usually accepted idea. The same can be shown for complement.

The first to draw attention to the true method of complement action was Scheller (1910[1]). We independently drew attention to the same fact (1912[2]). Scheller came to the conclusion that complement does not act quantitatively but by concentration, and that in this way it could do an indefinite amount of work. Our investigations agree with these on certain points, but differ in others. In this paper we shall detail our investigations on

I. Mode of action of complement.
II. Mode of action of amboceptor.

---

[1] [Received August 27, 1914.] Part of the expenses of the research was defrayed by a grant from the Government Grant Committee of the Royal Society of London.

III. Effect on complement concentration with increasing amboceptor.
IV. Rate of hæmolysis and complement concentration.
V. The alteration in the complement titre with increasing concentration of the antigen.
VI. Effect of temperature.

In this paper the complement was derived from well and continuously fed guinea-pigs. The serum after separation was tested to see that it was not directly hæmolytic to sheep's red corpuscles. It was then diluted with the necessary amount (0·9 per cent. NaCl in aq. destillata) of normal saline before use.

The hæmolytic amboceptor was against sheep's red cells. It was obtained from rabbits which had been repeatedly injected intraperitoneally with six times carefully washed sheep's red corpuscles.

1. *Mode of Action of Complement.*

In these following experiments the unit of complement was first of all ascertained for 100 c.mm. of a 25 per cent. suspension of once sensitised red cells (= unit of red cells) in 1 c.c. of saline.

EXPERIMENT 1.—Showing that the Activity of Complement depends on its Concentration and not on the Total Amount present.

The complement unit was 20 c.mm. pure for a unit of once sensitised red cells.

Saline.	Complement, 30 c.mm. in each, giving Concentration per c.c.	Complement, 100 c.mm. in each, giving Concentration per c.c.	Complement, 200 c.mm. in each, giving Concentration per c.c.
1 c.c.	1·5 units	5 units	10 units
3 ,,	0·5 ,,	1·6 ,,	3·3 ,,
5 ,,	0·3 ,,	1·0 ,,	2·0 ,,
10 ,,	0·15 ,,	0·5 ,,	1·0 ,,
15 ,,	0·1 ,,	0·3 ,,	0·6 ,,
20 ,,	0·06 ,,	0·25 ,,	0·5 ,,

— = Complete hæmolysis.

The time taken in all these experiments was thirty minutes in the water-bath at 40° C. The saline was previously heated to this temperature.

[Experiment 2.

EXPERIMENT 2.—TITRE OF COMPLEMENT FOR A UNIT OF 25 PER CENT. RED CELLS ONCE SENSITISED IN 1 C.C. SALINE=15 C.MM. PURE.

In this experiment 75, 100, 200 c.mm. of complement were placed respectively into each of a series of tubes with saline, as below.

Saline.	Complement, 75 c.mm. in each Tube.	Complement, 100 c.mm. in each Tube.	Complement, 200 c.mm. in each Tube.
	Units per c.c.	Units per c.c.	Units per c.c.
1 c.c. . .	5	6·6	13·3
3 ,, . .	1·6	2·2	4·4
5 ,, . .	1·0	1·3	2·6
10 ,, . .	0·5	0·6	1·3
15 ,, . .	0·3	0·4	0·9
20 ,, . .	0·25	0·2	0·6

—— = complete hæmolysis. …… = almost complete hæmolysis.

Numerous other similar experiments brought out the same fact, namely, that the complement activity depends on its concentration, and not on the total amount present.

EXPERIMENT 3.—THIS EXPERIMENT SHOWS IN ANOTHER WAY THAT COMPLEMENT WORKS BY CONCENTRATION AND NOT BY TOTAL AMOUNT.

The series of tubes were arranged containing 1, 2, 3, 5, 10, 15, and 20 c.c. of saline. Into each tube were placed a unit of once sensitised red corpuscle suspension (100 c.mm. of 25 per cent.) and graded amounts of complement. The tubes were then incubated in the water-bath at 40° C. for thirty minutes and the readings taken. The unit of complement in 1 c.c. was 20 c.mm. pure.

Complement, 50 per cent.	1 c.c.	2 c.c.	3 c.c.	5 c.c.	10 c.c.	15 c.c.	20 c.c. Saline.
20 c.mm.	M.H.						
30 ,,	V.M.H.						
40 ,,	C.H.	O.H.					
50 ,,	C.H.	S.H.					
60 ,,	C.H.	M.H.					
70 ,,	C.H.	A.C.H.	O.H.				
80 ,,	C.H.	C.H.	S.H.				
90 ,,	C.H.	C.H.	S.H.				
100 ,,	C.H.	C.H.	M.H.	S.H.			
120 ,,	...	...	C.H.	M.H.			
160 ,,	...	...	C.H.	V.M.H.			
200 ,,	...	...	C.H.	C.H.			
240 ,,	...	...	...	...	S.H.		
280 ,,	...	...	...	...	M.H.		
320 ,,	...	...	...	...	M.H.		
360 ,,	...	...	...	...	V.M.H.		
400 ,,	...	...	...	...	C.H.	S.H.	O.H.
500 ,,	...	...	...	...	C.H.	S.H.	S.H.
600 ,,	...	...	...	...	C.H.	C.H.	M.H.
700 ,,	...	...	...	...	...	C.H.	V.M.H.
800 ,,	...	...	...	...	...	C.H.	C.H.
900 ,,	...	...	...	...	...	C.H.	C.H.
1000 ,,	...	...	...	...	...	C.H.	C.H.

|‾‾‾| indicates the unit of complement for the dilutions in question.

It will be seen on analysis that the amounts of complement required to produce hæmolysis in the standard time were direct multiples of the amount required in 1 c.c., so that the complement concentration necessary to produce complete hæmolysis is the same for each dilution.

	Total Quantity.	Concentration per c.c.	
Thus for 1 c.c. .	= 40 c.mm. 50 per cent.	40	c.mm. (50 per cent.) = 1 unit per c.mm.
,, 2 ,, .	= 80 ,, ,,	40	
,, 3 ,, .	=120 ,, ,,	40	
,, 5 ,, .	=200 ,, ,,	40	
,, 10 ,, .	=400 ,, ,,	40	
,, 15 ,, .	=600 ,, ,,	40	
,, 20 ,, .	=800 ,, ,,	40	

These experiments, therefore, conclusively show that for red cells sensitised with one unit of amboceptor, complement does not work by absolute quantity, but by concentration.

II. *The Mode of Action of the Amboceptor.*

In the following experiments it will be shown that amboceptor does not combine with the red cells according to the degree of concentration, but according to the total amount, *i.e.*, the number of amboceptor units.

EXPERIMENT 4.

The unit of amboceptor was determined for 100 c.mm. of 25 per cent. red cell suspension in 1 c.c. saline by a "simultaneous estimation" of amboceptor and complement (Thiele and Embleton, 1914[3]). Then a series of tubes was taken containing different amounts of saline, 100 c.mm. of the red cell suspension added, and the determined unit of amboceptor. The contents of the tubes were then thoroughly mixed together and allowed to stand at 40° C. for thirty minutes; at the end of that time the contents of each tube were centrifugalised, the deposit of red cells taken up in 1 c.c. of saline, and the unit of complement added. These tubes were then incubated for thirty minutes at 40° C., and the results taken.

Thus:

100 c.mm. 25 per cent. red cells and one unit amboceptor added to

Saline c.c.	1	2	3	4	5	10	15	20	30	50
Amboceptor concentration in units	1	0·5	0·3	0·25	0·2	0·1	0·06	0·05	0·03	0·02

incubated for thirty minutes, centrifugalised, and then the deposit made up to 1 c.c. and the unit of complement added.

Complete hæmolysis occurred in all the tubes.

Numerous other experiments gave identical results, the times of preliminary treatment in the dilution varying from ten to sixty minutes. These experiments show conclusively that amboceptor does not act by its concentration, but combines with the red cells, no matter what the dilution. The activity of the amboceptor thus depends on its total amount and not on its concentration.

The same thing can be determined in another way by—

1. Accurately determining the unit of amboceptor and complement in 1 c.c. saline for 1 unit of red cells.
2. Arranging two series of tubes (*a* and *b*) containing various amounts of saline and putting into (*a*) once sensitised corpuscles, which after exposure to the amboceptor were centrifugalised and washed; (*b*) the unit of corpuscles and unit of amboceptor separately.
3. Adding to each the appropriate amount of complement for the dilution (*vide supra*) and noting if the hæmolysis is parallel in the two series.

EXPERIMENT 5.

		Series A.		Series B.	
Saline.	Complement pure.	Sensitised Corpuscles.		Amboceptor Concentration in Unit per c.c. (Components added separately).	
1 c.c.	20 c.mm.	1 unit.	Complete hæmolysis all through in 20 minutes	1·0	Complete hæmolysis all through in 20 to 24 minutes.
2 ,,	40 ,,	,,		0·5	
3 ,,	60 ,,	,,		0·3	
4 ,,	80 ,,	,,		0·25	
5 ,,	100 ,,	,,		0·2	
10 ,,	200 ,,	,,		0·1	
15 ,,	300 ,,	,,		0·06	
20 ,,	400 ,,	,,		0·05	
50 ,,	1000 ,,	,,		0·02	
100 ,,	2000 ,,	,,		0·01	

EXPERIMENT 6.—Similar Experiment.

		Series A.	Series B.
Saline.	Complement.	100 c.mm. Red Cells, sensitised with 1 Unit of Amboceptor.	100 c.mm. Red Cells. One Unit of Amboceptor added separately.
1 c.c.	20 c.mm.		
2 ,,	40 ,,	Hæmolysis was equally complete and rapid all the way through in sixteen minutes. The unit of complement was 15 c.mm. Hence each tube had 1·3 units per c.c.	
3 ,,	60 ,,		
4 ,,	80 ,,		
5 ,,	100 ,,		
6 ,,	120 ,,		
7 ,,	140 ,,		
8 ,,	160 ,,		
9 ,,	180 ,,		
10 ,,	200 ,,		
11 ,,	220 ,,		
12 ,,	240 ,,		
13 ,,	260 ,,		
14 ,,	280 ,,		
15 ,,	300 ,,		

These experiments again show that the activity of the amboceptor does not depend on its concentration, but on the total amount present.

III. *Effect of increasing Quantities of Amboceptor on the Complement Titre and Concentration necessary for Hæmolysis.*

In these experiments 100 c.mm. of 25 per cent. suspension of sheep's red cells were placed in different amounts of saline and sensitised with different amounts of amboceptor, and the quantity and concentration of complement determined which gave complete hæmolysis in thirty minutes under the previously mentioned conditions. The unit of amboceptor was carefully ascertained by a simultaneous estimation of complement and amboceptor (Thiele and Embleton, 1914 [3]).

EXPERIMENT 7.

Saline.	Amboceptor Units.	1	2½	5	10	50
1 c.c. . .	Quantity of complement in c.mm. necessary for complete hæmolysis in thirty minutes.	40	30	30	30	30
3 ,, . .		120	40	30	30	30
5 ,, . .		200	60	50	40	30
10 ,, . .		400	75	60	60	40
15 ,, . .		600	120	75	75	60
20 ,, . .		800	150	100	100	80
30 ,, . .		1200	250	150	150	120
50 ,, . .		2000	400	250	250	200

The complement concentration per c.mm. of this experiment is given in Table I.

TABLE I.

Saline.	Complement Concentration per c.c. in c.mm.				
	Amboceptor Units.				
	1	2½	5	10	50
1 c.c.	40	30	30	30	30
3 ,, ...	40	13	10	10	10
5 ,, ...	40	12	8	8	6
10 ,, ...	40	7·5	6	6	4
15 ,, ...	40	8	5	5	4
20 ,, ...	40	7·5	5	5	4
30 ,, ...	40	8	5	5	4
50 ,, ...	40	8	5	5	4

Numerous other experiments gave almost identical results.

Thinking that the "peculiarities" in complement concentration in the lower dilution with multiple units of amboceptor might depend on the use of 25 per cent. corpuscular suspension, we repeated the experiment with 10 per cent. suspension of red cells, with identical results.

EXPERIMENT 8.—100 c.mm. of 10 per cent. Red Cell Suspension.

Saline.		Amboceptor Units.				
		1	2½	5	10	50
1 c.c.	Complement in c.mm.	20	10	10	10	10
2 ,, ...	,, ,,	40	10	10	10	10
3 ,, ...	,, ,,	60	15	10	10	10
5 ,, ...	,, ,,	100	25	15	15	10
10 ,, ...	,, ,,	200	50	30	30	20
15 ,, ...	,, ,,	300	75	45	45	30
20 ,, ...	,, ,,	400	100	60	60	40
30 ,, ...	,, ,,	600	150	90	90	60
50 ,, ...	,, ,,	1000	250	150	150	100

On analysis it will be seen that the result is identical with that for 25 per cent. red cell suspension. Table II. gives the complement concentration per c.mm.

TABLE II.

Saline.	Amboceptor Units.				
	1	2½	5	10	50
1 c.c.	20	10	10	10	10 c.mm. of complement.
2 ,, ...	20	5	5	5	5 ,, ,,
3 ,, ...	20	5	3·3	3·3	3·3 ,, ,,
5 ,, ...	20	5	3	3	2 ,, ,,
10 ,, ...	20	5	3	3	2 ,, ,,
15 ,, ...	20	5	3	3	2 ,, ,,
20 ,, ...	20	5	3	3	2 ,, ,,
30 ,, ...	20	5	3	3	2 ,, ,,
50 ,, ...	20	5	3	3	2 ,, ,,

These experiments, which have been frequently repeated with similar results, show that—

1. With 1 unit of amboceptor the true complement concentration can be ascertained in 1 c.c. dilution, and that the amount necessary for higher dilutions is a direct multiple of this.
2. With more than 1 unit of amboceptor, complement concentration does not become constant till a suitable

degree of dilution of the sensitised red cells has been reached. This varies for the different degrees of sensitisation, being greater the stronger the sensitisation. The meaning of this phenomenon will be discussed later.

3. When the suitable dilution has been reached, the complement works as with once sensitised red cells, by concentration, and for further dilutions the amounts of complement necessary are direct multiples of this (see Figs. 1–4).

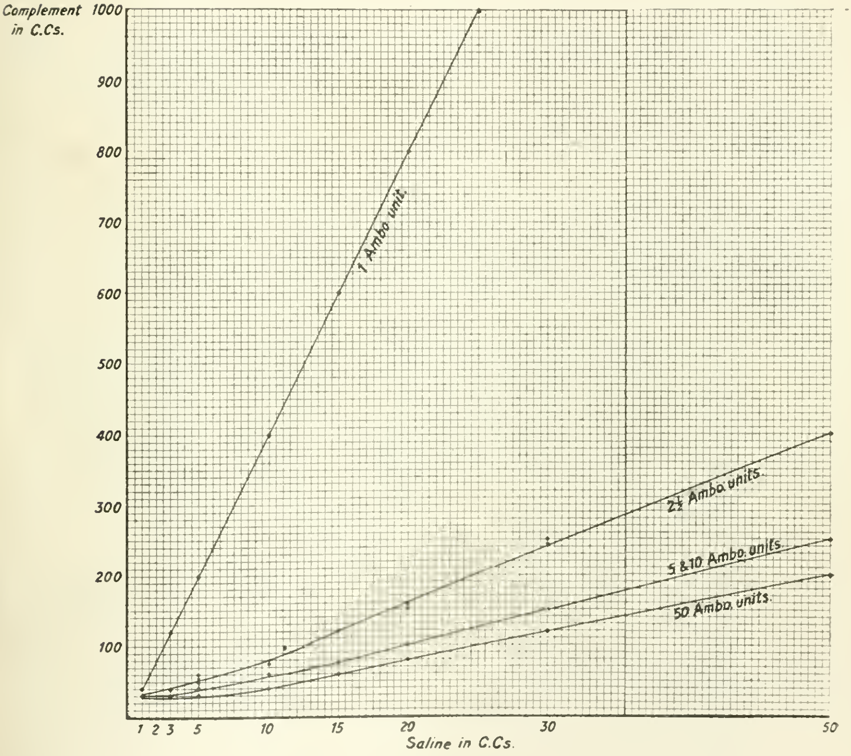


FIG. 1.

We have just drawn attention to the peculiarity that although with 1 unit of amboceptor the true minimal complement concentration necessary for complete hæmolysis can be obtained in 1 c.c. dilution, yet, with multiple doses of amboceptor, the true minimal complement concentration is only obtained when the cell-amboceptor mixture has been further diluted. The necessary degree of dilution varies for the specimen of amboceptor and for the number of units of amboceptor used, as will be seen from the following experiments.

Thus:

EXPERIMENT 9.—100 c.mm. 25 per cent. Red Cell Suspension in Various Quantities of Normal Saline, with Different Units of Amboceptor, the Complement Concentration necessary for Complete Hæmolysis was determined.

Saline.	Amboceptor Units.				
	1	2½	5	25	
1 c.c.	20	20	20	24	Complement concentration in c.mm. necessary for complete hæmolysis.
2 ,,	20	16	10	8	
5 ,,	20	8	6	6	
10 ,,	20	8	4	4	
20 ,,	20	8	4	4	

From this experiment it will be seen that with 2½ units of amboceptor, the minimal concentration is reached in 5 c.c. dilution, whilst for the 5 and 25 it is only reached in the 10 c.c. dilution.

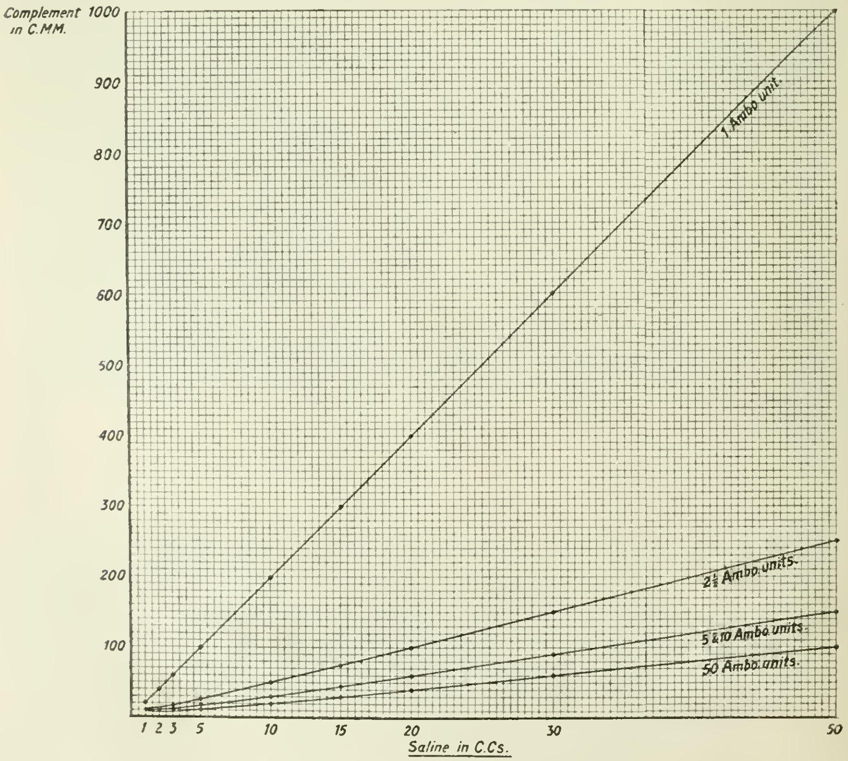


Fig. 2.

EXPERIMENT 10.—Same Experiment as Experiment 9, but using a 10 per cent. Red Cell Suspension.

Saline.	Complement Concentration per c.c. in c.mm. pure.				
	Amboceptor Units.				
	1	2½	5	10	50
1 c.c. . .	20	10	10	10	10
2 ,, . .	20	5	5	5	5
3 ,, . .	20	5	3·3	3·3	3·3
5 ,, . .	20	5	3	3	2
10 ,, . .	20	5	3	3	2
20 ,, . .	20	5	3	3	2

In this experiment the amount of complement remains constant with the 5 and 10 units of amboceptor till the 3 c.c. dilution, with the 50 till the 5 c.c. dilution, but with the 2½ units only till the 2 c.c. dilution.

EXPERIMENT 11.—Same Experiment, using a 25 per cent. Red Cell Suspension.

Saline.	Complement concentration per c.c. in c.mm. pure.				
	Amboceptor Units.				
	1	2½	5	10	50
1 c.c. . .	30	20	20	20	20
2 ,, . .	30	10	10	10	10
3 ,, . .	30	6·6	6·6	6·6	6·6
5 ,, . .	30	8	6	6	4
10 ,, . .	30	8	6	6	4
20 ,, . .	30	8	6	6	4

In this experiment the total amount of complement remains constant with the 2½ units of amboceptor till after the 3 c.c. dilution, with the 5 and 10 units of amboceptor till after the 3 c.c. dilution as well, with the 50 units of amboceptor till after the 5 c.c. dilution.

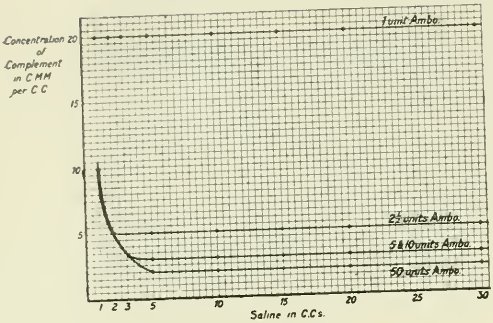


FIG. 3.—10 per cent. red cell suspension.

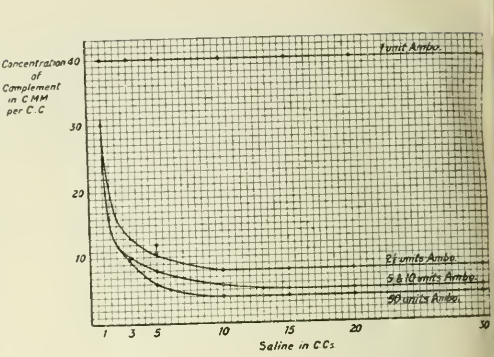


FIG. 4.—25 per cent. red cell suspension.

The following experiments show the same points.

In these 25 per cent. corpuscular suspension was used (100 c.mm.).

EXPERIMENT 12.

Saline.	Complement Concentration per c.c. in c.mm.						
	Amboceptor Units.						
	1	5	10	15	20	50	100
1 c.c. . . .	24	24	24	24	24	24	24
10 ,, . . .	20	6·4	5	4	4	4	4

EXPERIMENT 13.

Saline.	Complement Concentration per c.c. in c.mm.					
	Amboceptor Units.					
	1	5	10	15	20	50
1 c.c.	30	30	30	30	30	30
2½ ,, ...	30	12	7	7	7	7
10 ,, ...	30	9	5	4	4	5

These experiments bring out the same point with both 10 per cent. and 25 per cent. red cell suspension.

(*a*) In 1 c.c. dilution, increasing amboceptor units only slightly diminish the minimal dose of complement. The difference is greater with the 10 per cent. than with the 25 per cent. suspension.

(*b*) The true minimal concentration is only apparent on dilution, and the degree of dilution necessary varies with the amboceptor dose and the particular amboceptor.

It would thus appear, as has already been brought out, that a certain minimal amount of complement must be present before the reaction can manifest itself, and that this minimal amount for 1 c.c. dilution may be equal to that necessary to make the suitable concentration for higher dilutions.

This same point is shown very effectually by the following experiments:—

EXPERIMENT 14.—THE COMPLEMENT CONCENTRATION WAS DETERMINED FOR 1 UNIT (100 C.MM.) OF 25 PER CENT. RED CELL SUSPENSION WITH 10 UNITS OF AMBOCEPTOR IN 5 AND 10 C.C. OF SALINE, AND WAS FOUND TO BE 6 C.MM. PURE.

In 1 c.c. with 10 units of amboceptor it was 30 c.mm. pure. Complement was then added, 6 c.mm. per c.c. of saline, for the following quantities of saline, to which the unit of red cells and 10 units of amboceptor had been added. The results were as follows:—

Saline, c.c.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Complement c.mm.	6	12	18	24	30	36	42	48	54	60	66	72	78	84	90
Result .	OH	OH	SH	MH	ACH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH

← The order of lysis was in this direction. ←

EXPERIMENT 15.—AS ABOVE, WITH 50 UNITS OF AMBOCEPTOR.

In 10 c.c. the complement concentration per c.c. was for . .	1	5	10	20	50 units of amboceptor.
	25	10	6	6	6 complement concentration per c.c. in c.mm.

The unit concentration of complement per c.c. was added for each c.c. to the following quantities of saline. One unit of red cells and 50 units of amboceptor had been previously put in.

Saline, c.c.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Complement c.mm.	6	12	18	24	30	36	42	48	54	60	66	72	78	84	90
Result .	MH	MH	ACH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH	CH

Overnight. In thirty minutes—largest complement quantity acting first.

These experiments show definitely that a minimal quantity of complement must be present, without which complete hæmolysis cannot occur, no matter how great the degree of amboceptor activity. Thus, in Experiment 14, complete hæmolysis in thirty minutes only occurred down to the tubes containing 30 to 36 c.mm. of complement, *i.e.*, the amount which was necessary to cause complete hæmolysis in 1 c.c.

In Experiment 15, complete hæmolysis occurred down to the tube containing 30 c.mm., *i.e.*, the tube containing about the amount of complement which was necessary to cause complete hæmolysis in 1 c.c. (25 c.mm.). If, however, the unit concentration per c.c. used, is the unit as determined for 1 unit of amboceptor in 1 c.c. of saline, then the hæmolysis is even and uniform in time.

EXPERIMENT 16.—THUS WITH 25 PER CENT. CORPUSCLE SUSPENSION AND 1 UNIT OF AMBOCEPTOR, THE COMPLEMENT TITRE WAS FOUND TO BE 20 C.MM. PURE, REPEATING THE SAME PROCEDURE AS BEFORE.

Saline c.c.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Complement c.mm.	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300
Hæmolysis even and complete all through practically simultaneously.															

It thus becomes quite apparent that—

(*a*) There must be present in order to produce complete hæmolysis a minimal quantity of complement, and that with red cells sensitised with 1 unit of amboceptor, this corresponds to the unit concentration per c.c.

(*b*) With more than 1 unit of amboceptor, the minimal quantity will still continue to exert its activity, provided that it is present in the concentration necessary for that dilution; if not, so much more complement is required till the required concentration is reached. This varies with the degree of amboceptor sensitisation and the amboceptor under consideration.

It thus appears that a minimal quantity of complement is necessary for each corpuscle to secure complete hæmolysis. The action of the amboceptor is to increase the area of the zone around each corpuscle in which complement activity can exert itself, so that the same amount of complement will work even when its concentration is diminished, until the limit of the increased zone of action brought about by virtue of the increased amboceptor is reached. After that more complement has to be added to bring the concentration up to the required amount. It is also apparent that the complement working in this way does not go into quantitative combination.

IV. *Influence of Complement Concentration Units and increasing Amboceptor on the Rate of Reaction.*

EXPERIMENT 17.—CORPUSCLES SENSITISED WITH 1 UNIT OF AMBOCEPTOR.
Showing the effect of varying quantities of complement.
All done in room incubator at 37° C. All materials were at 37° C. before starting the experiment.

Saline.	1 Unit in each Tube.		1·5 Units per c.c. in each.		100 c.mm. (5 Units in each).		200 c.mm. (10 Units in each).	
	Compl. Conc. Units.	Time.*	Compl. Conc. Units.	Time.*	Compl. Conc. Units.	Time.*	Compl. Conc. Unit.	Time.*
1 c.c.	1	28 min.	1·5	11 min.	5·0	2 min.	10·0	1·5 min.
3 ,,	1	28 ,,	1·5	11 ,,	1·6	9 ,,	3·3	5 ,,
5 ,,	1	28 ,,	1·5	11 ,,	1·0	24 ,,	2·0	7 ,,
10 ,,	1	28 ,,	1·5	11 ,,	0·5	61 ,,	1·0	24 ,,
15 ,,	1	28 ,,	1·5	11 ,,	0·3	overnight	0·6	40 ,,

* For complete hæmolysis.

EXPERIMENT 18.—Showing effect of increasing the Amboceptor.
100 c.mm. of 25 per cent. corpuscle suspension in 10 c.c. saline.
All done in room incubator at 37° C., as above.

Complement 1–5.	Concentration per c.c.		Amboceptor Units.			
	(1–5)	Pure.	1	5	10	50
			Time.*	Time.*	Time.*	Time.*
50 c.mm.	5	1	...	...	...	...
100 ,,	10	2	...	...	120 min.	...
150 ,,	15	3	...	...	50 ,,	40 min.
200 ,,	20	4	...	...	21 ,,	10·5 ,,
300 ,,	30	6	...	...	1 ,,	4 ,,
400 ,,	40	8	...	80 min.	5 ,,	2·5 ,,
500 ,,	50	10	...	45 ,,	4·5 ,,	1
600 ,,	60	12	...	20 ,,	...	...
800 ,,	80	16	...	9 ,,	...	...
1000 ,,	100	20	90 min.	8 ,,	...	...
1500 ,,	150	30	30 ,,	4 ,,	...	...

* For complete hæmolysis. The unit concentration, according to standard taken for each, is indicated by the quantity corresponding to the time underlined.

EXPERIMENT 19.—Similar to previous one. In 10 c.c. Saline.

Complement (1–5).	Complement Concentration pure.	Amboceptor Units.				
		1	5	10	20	50
		Time in Minutes.				
100 c.mm. .	2 c.mm.	...	...	...	...	...
200 ,, .	4 ,,	...	...	Not in 30	Not in 30	Not in 30
300 ,, .	6 ,,	...	...	18	13	6
400 ,, .	8 ,,	...	...	12	7	4
500 ,, .	10 ,,	...	Not in 30	8	5	2
600 ,, .	12 ,,	...	20	4	3	1
700 ,, .	14 ,,	...	14	2	1	1
800 ,, .	16 ,,	Not complete overnight	10	1	1	1
900 ,, .	18 ,,	...	6	1	1	1
1000 ,, .	20 ,,	90	4	1	1	1
1500 ,, .	30 ,,	30	3	1	1	1

This experiment shows that although increasing amboceptor increases the rate of hæmolysis, yet the complement titre remains stationary after the amboceptor has been increased beyond a certain point. Thus, whereas in the experiment the time for complete hæmolysis gradually got shorter the greater the amount of amboceptor, the complement titre remained stationary with 10, 20, and 50 units of amboceptor.

To determine the Rate of Hæmolysis of Red Cells, sensitised once, with varying Degrees of Complement Concentration.

A series of tubes, each containing 9 c.c. of saline, was kept in the water-bath at 40° C. till they had attained that temperature. Then to each was added 1 c.c. of six times washed sheep's red cells, the appropriate amount of amboceptor to sensitise the corpuscles once, and complement so that the complement concentration was 5, 2, 0·8, 0·5 and 0·3 units per c.c. Specimens, 1 c.c. mixture, were taken out at the end of every five minutes, cooled, centrifugalised, the deposit collected and distilled water added to 1 c.c. The tint was then compared with a series of tubes containing 5, 10, 15, 20, 30, etc., c.mm. of the red cell suspension (hæmolysed in 1 c.c.), and the reading taken of the amount of the cells unhæmolysed.

EXPERIMENT 20.—Complement Units per c.c.

Time	5	2	1	0·8	0·5	0·3
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
5 minutes	100	95	45	30	25	15
10 ,,	...	100	65	45	35	25
15 ,,	...	...	80	55	50	30
20 ,,	...	...	90	80	60	45
25 ,,	...	...	95	85	75	55
30 ,,	...	...	100	90	80	60
35 ,,	...	...	...	90	85	60
40 ,,	...	...	...	95	90	60
45 ,,	...	...	...	100	90	60
50 ,,	...	...	...	...	90	65
60 ,,	...	...	...	...	90	70

Per cent. of hæmolysis.

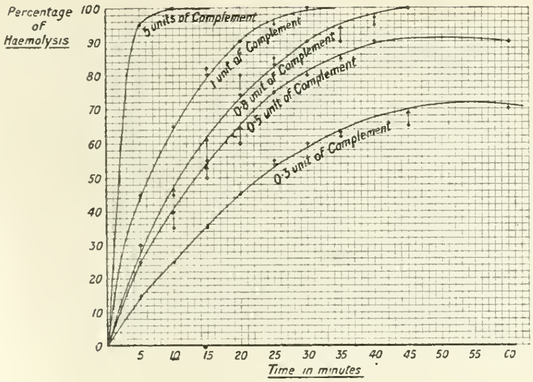


FIG 5.

This is plotted out in the accompanying curve (Fig. 5), and the curve has the general appearance of a similar curve plotted for true enzyme action, and appears like a unimolecular curve.

Other experiments gave similar results.

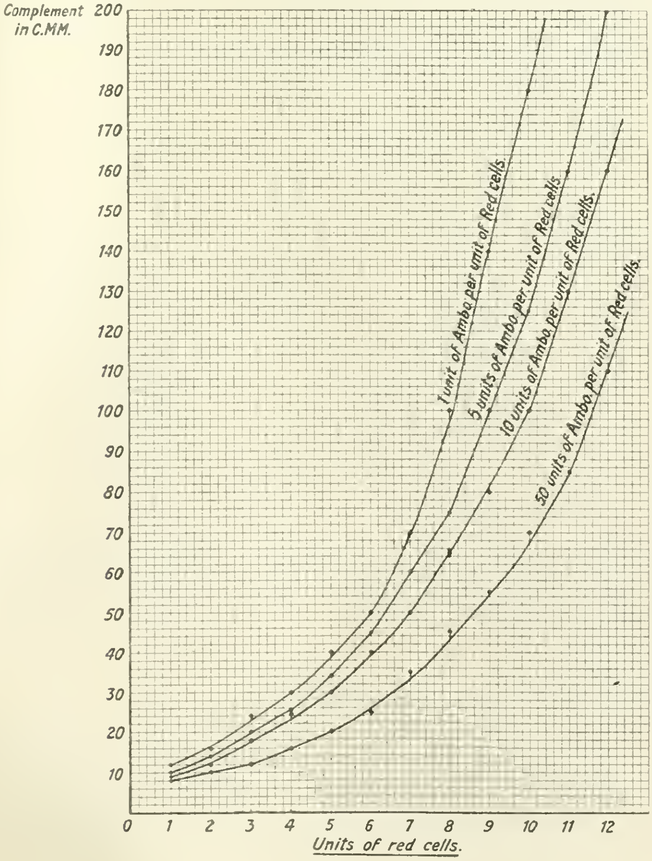


FIG. 6.

V. *The Effect of increasing the Antigen Concentration.*

A series of experiments was performed to find the quantity of complement necessary to cause complete hæmolysis of varying quantities of red cells sensitised with varying strengths of amboceptor in a constant dilution. 100 c.mm. of a 10 per cent. red cell suspension in normal saline were taken as the unit of red cells. The unit of amboceptor was found by the simultaneous method of amboceptor and complement, all tubes being centrifugalised for the purpose of taking the reading, thus ensuring a very accurate approximation to the absolute unit. The minimum of complement necessary to cause complete hæmolysis of 1 unit of red cells sensitised with unit of amboceptor in a total dilution of 1 c.c. was found. Then the quantity of complement for 2 units of red cells, each unit sensitised with 1 unit of amboceptor, total bulk 1 c.c., then 3 units of red cells, each unit sensitised with 1 unit of amboceptor, etc., up to 10 units of red cells, was found. After this the minimum quantity of complement to produce hæmolysis of 1, 2, 3, etc., units of red cells, each unit sensitised with 5 units of amboceptor, was found. And so on for ten times and fifty times sensitised units. The results appear in Experiment 21, but are best seen by referring to Fig. 6.

EXPERIMENT 21.—TOTAL QUANTITY IN EACH TUBE 1 C.C.

Units of Amboceptor per unit Red Cells.	Units of Red Cells.											
	1	2	3	4	5	6	7	8	9	10	11	12
1	12*	16	24	30	40	50	70	100	140	180	not in 200	...
5	10	14	20	25	35	45	60	75	100	125	160	200
10	9	12	18	25	30	40	50	65	80	100	130	160
50	8	10	12	16	20	25	35	45	55	70	85	110

* Least quantity in c.mm. of complement necessary to cause complete hæmolysis. For obtaining these titres, the intervals between consecutive quantities were as follows:—5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 25, 30, 35, 40 c.mm., and so on by increments of 5 c.mm. to 200 c.mm.

From these experiments it is obvious that—

1. The complement concentration necessary to cause hæmolysis of 1 unit of red cells is insufficient to cause complete hæmolysis of more than 1 unit in the unit of time. So that in a unit of time a given complement concentration can only do a certain amount of work.

2. The increase in the amount of complement necessary to do increasing amounts of work in the unit time is not proportional to the increase in the work done, *i.e.*, for 2 units or 3 units of work, it is not twice or thrice that concentration required for 1 unit.
3. The increase necessary for the first few units extra is in fractions of the concentration necessary for unit hæmolysis; later it increases by larger fractions, and with larger quantities still it increases by amounts greater than the original unit concentration. This can be seen from the following summary, which is an analysis of Experiment 21.

Units of Amboceptor.	Units of Red Cells.											
	1	2	3	4	5	6	7	8	9	10	11	12
	Additional complement required for each successive unit of red cells in c.mm.											
1	12	4	8	6	10	10	20	30	40	40	...	...
5	10	4	6	5	10	10	15	15	25	25	35	40
10	9	3	6	7	5	10	10	15	15	20	30	30
50	8	2	2	4	4	5	10	10	10	15	15	25

4. Since the complement necessary, therefore, does not increase after the first units by direct multiples of the unit, and as the additional amount increases out of proportion to the extra work to be done, it must follow that something is developed as the result of the amboceptor-complement activity which exercises an anti-complementary effect, which becomes more marked the greater the concentration of this anti-complementary substance.
5. This anti-complementary power is not due wholly, anyhow, to the absorption of the complement by the formation of a precipitate. The precipitate occurs most markedly and rapidly with the larger doses of amboceptor, but the complement proportion per unit of work rises most rapidly the less the amboceptor.

Further investigations are in progress on the latter point and anti-complementary phenomena in general. We would therefore conclude that complement works by its concentration, and this is diminished within limits the greater the amount of amboceptor present.

Complement working under the ordinary conditions does not do an unlimited amount of work; with slightly increased quantities of antigen to be attacked, the increase is slight; later the increased amount required is excessive, and this probably depends on the inhibitory effects of the products of the ferment action. Complement does not go into quantitative combination with the antigen, and only little combines or is used up in the reaction, as will be seen from the small increasing amounts necessary to do increased work in standard time till the complement is interfered with by other factors. Amboceptor combines quantitatively with its homologous antigen, and acts as a determinant and accelerator of complement action. Complement follows the laws of fermentation.

VI. *Showing the Effect of Temperature on Complementary Activity.*

EXPERIMENT 22 PERFORMED AS ABOVE.

100 c.mm. of a 10 per cent. red cell suspension in 1 c.c. of saline.

	Temperature of Water-bath.	Complement Dilution in c.mm.				
		100	80	60	40	20
Amboceptor, 1 unit .	40° C.	8½*	9	14	–	–
	35° C.	11	14	16	–	–
	30° C.	15	16	21	–	–
Amboceptor, 10 units	40° C.	2	2½	3¾	8½	–
	35° C.	3½	4	5½	10	–
	30° C.	4½	5½	7	15	–

* Time in minutes for complete hæmolysis of 1 unit of red cells in 1 c.c.
– Complete hæmolysis did not occur in three hours.

In the limits of the experiment the difference in temperature did not alter the complement titre but the rate of reaction. At lower temperature, however, the complement titre is affected.

SUMMARY.

1. The activity of complement depends solely on its concentration, not on the total amount exhibited.

This holds all through when the antigen has been sensitised with only one dose of antibody. When, however, the antigen has been sensitised with several doses, the above holds after a certain degree of dilution. This degree of dilution varies with the amount of and individual antibody.

2. Amboceptor combines quantitatively with its homologous antigen, and its activity does not depend on its concentration.

3. Increasing amboceptor diminishes to a certain degree the complement concentration necessary to produce complete lysis. Above a certain amount increase in the amount of amboceptor does not diminish the necessary complement concentration. It, however, greatly accelerates the rate of the reaction.

4. In addition to requiring a certain concentration, complement must also be present in a certain minimal amount. It thus frequently occurs that the amount of complement necessary to produce complete lysis is the same in dilutions of 1, 2, 3 and more c.c.

5. Study of the curves of complement activity shows that they are like those of enzyme action.

6. With increasing concentration of the antigen more complement is necessary to produce complete lysis in standard time. This is opposed to the results of Scheller.

The necessary increase is at first small, a fraction of the original unit; later, the increase with each successive dose of antigen is much greater, and ultimately becomes greater than, and a multiple of, the original unit.

REFERENCES.

1. SCHELLER . . . . . . *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1910, Bd. lvi. S. 120.
2. THIELE AND EMBLETON . . "Pathogenicity and Virulence of Bacteria," *Proc. Roy. Soc. Med.*, London, 1912, Pt. 3, Path. Sec. 13, vol. vi. p. 99.
3. THIELE AND EMBLETON . . *Lancet*, London, 1914, vol. i. p. 1032.

Klossiella muris.

By
A. C. Stevenson, M.B., D.P.H.,
University College Hospital Medical School.

With Plate 10.

This parasite was first described by Smith and Johnson in 1902. There are only two other papers I know of on the subject, one by Woodcock of a critical nature, and one by Sangiorgi containing experimental facts of interest.[1] In all the above the only phases of the parasite described and mentioned are those in the glomeruli of the kidney, the epithelium of the tubules, and the urine. The first describers consider the schizogony that takes place in the glomeruli as a formation of merozoites, and the tubular phase as sporogony leading on to spores and sporozoites in the urine. Woodcock, on the analogy of Caryotropha mesnili, suggests that the tubular phase is one of schizogony leading to merozoites and that possibly the glomerular phase is one of schizogony leading to gametocytes. Sangiorgi, in a paper of which I have received an abstract from Woodcock, holds the same view with regard to the tubular phase, and says that he has infected mice with urine which contained cysts, but he seems unaware that these cysts contained definite products of division. Sangiorgi also states that he could not infect mice from mash of kidney-substance, and states that he considers the cysts in the urine to be oöcysts and describes the glomeruli of the kidney as containing bodies which he describes as sporozoites in a membrane or spore-capsule. He says that difficulty arises as to the phase of the parasite in which a new host is infected.

---

[1] In addition to these Seidelin has lately published a paper describing a similar parasite in the guinea-pig in Africa, but mentions no other forms than those found by Smith and Johnson.

As will be seen, I consider the original describer is right in thinking the stage in the tubules to be sporogony, since cysts found freely in the urine of infected mice are practically in the same condition as those seen in the last stages of development in the kidneys. Sangiorgi's first experiment confirms this, but a criticism on the experiment must be raised as to whether his mice were previously infected or not. Examination of the urine would prove nothing in the early stages. I have several times missed infections in fresh specimens of the kidney, and found the parasite when I cut sections, cysts being nowhere present.

If the cysts are not spores, it is difficult to imagine how another host is to be infected on the analogy of coccidium. If cysts contain gametocytes and an insect host is concerned, there is only one I have heard of that is definitely attracted by urine, ants.

The schizogony in the glomeruli I consider to be one that produces gametocytes and to be secondary to the one producing merozoites which takes place elsewhere.

My great difficulty is the type of syngamy that takes place, but I have no doubt that this stage takes place in the cells of the kidney tubules.

I have found the parasite in about 40 per cent. of the white mice examined (25). I have an impression that it occurs more frequently during the summer months, but this may be due to differences in the source of supply of the mice. It is, however, possible that infection is brought about by an invertebrate host prevalent in summer, and, from what I have seen of the organism, I think this may take place as well as infection by the normal casual coccidial method.

Forms observed.

What is possibly the earliest form I have seen was in a mouse, which post-mortem showed no parasites in the kidney, but had lived in a cage with others that were passing cysts. It consisted of a small gregariniform body about 12 μ long, free in the peripheral blood (Pl. 10, fig. 1). When stained by Giemsa, however, its morphology very closely resembles that of the merozoites of an intestinal coccidium of the mouse, and it is quite possible that one of these might have found its way into the blood-stream. I do not know if this mouse had coccidiosis of the intestine, but a very large percentage have.

The next stage I have seen was one in an arteriole of the kidney (Pl. 10, fig. 2). This consisted of a round body in an endothelial cell of the arteriole, with three nuclei in process of division. The diameter is about 8 μ. It may, however, be an early instance of either of the following types of schizogony.

In a similar position I have found a parasite definitely divided up into eight to twelve daughter individuals, these still being contained in an envelope formed by the remains of the host-cell (Pl. 10, fig. 3). This I take to be the schizogony of the parasite into merozoites. I have searched many sections of kidneys and other organs both in infected and uninfected mice, but up to the present I have not come across any other instances of the above stages. The daughter individuals in the above case have a definite nucleus with a well-marked karyosome. As the division in the other type of schizogony and also that into sporozoites are characterised by the great numbers of individuals produced, it is possible that this first type is comparatively infrequent in occurrence.

The second type of schizogony is very different, the daughter individuals being very numerous, fifty to sixty being a moderate estimate. This division takes place usually in the cells forming the capillaries in the glomeruli of the kidneys, but on rare occasions (three)[1] I have seen it occurring in the endothelial cells of the arterioles of those organs (Pl. 10, figs. 9, 10).

As first seen, the parasite in this stage is a small uninucleate body bulging into the capillary, the granules in it giving it a greenish-grey tinge when stained with iron-

---

[1] Since the above was written, I have seen two other instances; in one the parasite had only two nuclei, in the other division was complete.

hæmatoxylin and orange (Pl. 10, figs. 4, 5, 6). This nucleus divides apparently fairly rapidly till we get a large mass occupying quite a large portion of Bowman's capsule in section, and containing nuclei too numerous to count easily, but about fifty to sixty in number (Pl. 10, figs. 7, 8). Around these the protoplasm segments, and we get a large number of gregariniform bodies, each with a nuclear area, containing two small masses of chromatin, and some residual protoplasm (Pl. 10, figs. 11, 12, 13). These chromatin granules are marked by their ovoid shape, and lie side by side with their long axes parallel to that of the organism. The mass of merozoites lies in a capsule formed by the thinned-out remnants of the host-cell, and on the bursting of this into Bowman's capsule the organisms are set free to wander down the tubule of the kidney, where they enter the cells of the convoluted portion in, I believe, the condition of gametocytes or gametes.

The next stage is the one somewhat difficult to interpret. There is no doubt in my mind that the normal condition is a double infection of the cells, the two individuals being at first indistinguishable from one another. Single infections are also found, but I think the balance of evidence is that these do not develop farther in a normal way. There is certainly often found growth, which is sometimes marked, and even budding apparently occurs, but this latter is generally internal instead of the normal external type.

Again, in advanced cases where spores are freely found in the urine, one frequently finds cast-off epithelial cells containing uninucleate small parasites. Another point in favour of this view is that in tubules containing parasites well on in the sporoblastic stage and further, it is common to find cells containing small uninucleate parasites. When one considers that the parasites of the epithelium of the tubule must arrive there practically simultaneously, for it is rare to find two bodies undergoing schizogony in one Malpighian tuft, it is difficult to understand the great difference of development unless due to syngamy having occurred in the advanced cases.

What is the type of syngamy? There seem two possibilities. First, though the observed instances are not too numerous, that it resembles that of Adelea ovata,[1] where the nucleus of one of the two associated and closely applied individuals divides into microgametes (four), one of which fertilises the other individual.

Second, that complete fusion takes place between the cytoplasm of the individuals, and their nuclei divide, though fusion of the chromatin does not take place till a much later stage.

That anisogamy of the type of coccidium with many microgametes formed separately from the macrogametes does not take place, I am practically certain.

The evidence for either of the above two views is not very definite. In favour of the first are the facts that forms in one cell sometimes show differences of nucleus and cytoplasm, the one showing signs of division in one and not in the other, while the latter is more finely granular in the parasite with the dividing nucleus and coarse in the other (Pl. 10, figs. 17, 18, 19). In later stages when apparent fusion has taken place and division has proceeded, there can sometimes be observed a body closely resembling a bud, but more definitely separated from the main mass, which contains irregular masses of a chromatin-like staining substance (Pl. 10, figs. 22, 23, 26, 29).

In favour of the second view is the fact that all through the enlargement of the parasite after fusion there seems to be two types of chromatin present, shown by different staining reactions, the one retaining iron-hæmatoxylin well, and staining deeply with acid hæmalum, while in the other the staining intensity is less marked. These masses often lie close to one another in pairs, and the dark stained one seems to divide first. This condition is retained until the bud is finally separated from the mother individual. This, however, may be due to differences in the condition of the chromatin, division of nucleus, etc. (Pl. 10, figs. 26, 27).

Whichever of the above views is right, there is no doubt that fusion or association of two gametocytes takes place, and that after this nuclear division follows until there are twelve to sixteen nuclei arranged round the periphery of the parasite. The nuclei gradually travel outside the line of general contour of the parasite (Pl. 10, fig. 30) and along with some of the cytoplasm are finally budded off one by one. There can often be found a renal cell containing two or three buds and the remainder of the parasite still undivided (Pl. 10, fig. 31). By the time all the buds have formed, which are generally twelve to sixteen in number, the renal cell has become tremendously enlarged and often dilates the renal tubule to many times its original diameter. It is still, however, attached to the basement membrane by a fine point of apparently altered protoplasm. This fine attachment is seen at very early stages of infection of the cell; in fact, nearly all infected cells project freely into the lumen of the tubule (Pl. 10, figs. 15, 32).

---

[1] Further observations tend to this view.

The nuclei in these buds or sporoblasts then divide, enlargement takes place, and a cyst wall forms round the sporoblast till we finally get a spherical body containing about twenty-five nuclei, each of which latter consists of three granules of chromatin arranged in a line about an equator of the sphere and at right angles to the plane of that equator (Pl. 10, fig. 35). Around each three granules of chromatin the protoplasm segments, and we get a spore cyst containing about twenty-five sporozoites with a certain amount of residual matter left at one pole of the cyst (Pl. 10, figs. 36, 37, 38). The capsule containing these spores then bursts, and the spores travel down the tubules through the papilla and ureter to the bladder in which situations (except the ureter) I have found them in sections. In the urine they can be easily found if the infection is at all heavy.

General.

The later stages closely resemble those described by Christophers in the sporogony stage of Leucocytozoon

Canis in its invertebrate host. Miss Porter also in her description of the Leucocytozoon of the mouse mentions having found a schizogony stage in the bone-marrow. Is it possible that the organism we are dealing with is the same as this latter Leucocytozoon? We have only to postulate that in a large infection we might get a large number of parasites undergoing the gametocyte-forming schizogony in the arterioles, and when these were set free in the blood-stream they would probably be taken up by leucocytes. From Miss Porter's description the schizogony stage she saw was possibly that into merozoites.

Another interesting supposition also arises in regard to this parasite. Have we here a link between the hæmogregarines and the coccidia? We have only to presuppose that gametocyte formation takes place largely in the arterioles instead of in the Malpighian tuft capillaries, and we get the blood-stream charged with parasites in a condition suitable for sporogony in a blood-sucking host. The question of the greater (?) incidence of the parasite in summer also bears on this point.

From the medical standpoint of view the possibility of a protozoon being a parasite of the arteries seems to me to be of great importance.

METHODS.

Fresh specimens were always examined, the tissue being teased in normal saline. This was useful for general diagnosis, but slight or early infections might easily be overlooked.

Smears, after teasing, were also made and fixed by Schaudinn's method. These were good for small forms.

Most of the work was done with serial sections, the tissue being fixed with corrosive sublimate and glacial acetic. The staining of these and the smears was with iron-hæmatoxylin or acid hæmalum.

EXPLANATION OF PLATE 10,

Illustrating Mr. A. C. Stevenson's paper on "Klossiella muris.

REFERENCE LETTERS.

P. Parasite in its various forms. *C.* Host-cell or its remains. *N.* Nucleus of host-cell. *N*². Possible degenerate nucleus of host-cell. *R.* Red blood-corpuscles. *tn.* Nuclei of tissue-cells. *m.* Possible remains of microgametocyte. *Bc.* Bowman's capsule.

[Magnification × 1200.]

SCHIZOGONY CYCLE.

Fig. 1.—Free merozoite? in the blood-stream of a mouse which had been exposed to infection.

Figs. 2, *2a*, *2b*.—Young form in endothelial cell of kidney arteriole, showing division of nuclei. Three sections through parasite. This may be a form leading to that shown in fig. 3 or those in figs. 9 and 10.

Fig. 3.—Complete division of parasite into merozoites in endothelial cell of arteriole of kidney.

Figs. 4, 5, 6.—Small forms in the cells of capillaries of a glomerulus.

Fig. 7.—The same larger, nuclear increase.

Fig. 8.—Large form.

Figs. 9, 10.—Forms as in fig. 8, but with more nuclei, in the endothelial cells of the arterioles of a kidney.

Fig. 11.—Commencement of division forming gametes.

Fig. 12.—Complete division.

Fig. 13.—Same from a smear, showing residual protoplasm.

SPOROGONY CYCLE.

Figs. 14, 15, 16.—Showing double infection of the cells of the convoluted tubule of a kidney by small forms.

Figs. 17, 18, 19.—Double forms of larger size, showing slight differences in the cytoplasm and nucleus.

Figs. 20, 21.—Single forms in a cell.

Fig. 22.—Single form in a cell: early nuclear division. At one side of the parasite is a mass of homogeneous protoplasm containing chromatin granules possibly the remains of a microgametocyte. Similar masses are seen in figs. 23, 26, 29.

Fig. 23.—Form with two nuclei.

Figs. 24, 25.—Two sections through one parasite. Chromatin mostly in granules, some suggestions of a mitotic figure.

Figs. 26, 27.—Sections through two parasites in one tubule, both in the same section, showing differences of nuclear staining.

Figs. 28, 29, 30.—Increase of nuclei and commencement of budding.

Fig. 31.—Sporoblasts separating from the main mass of a parasite.

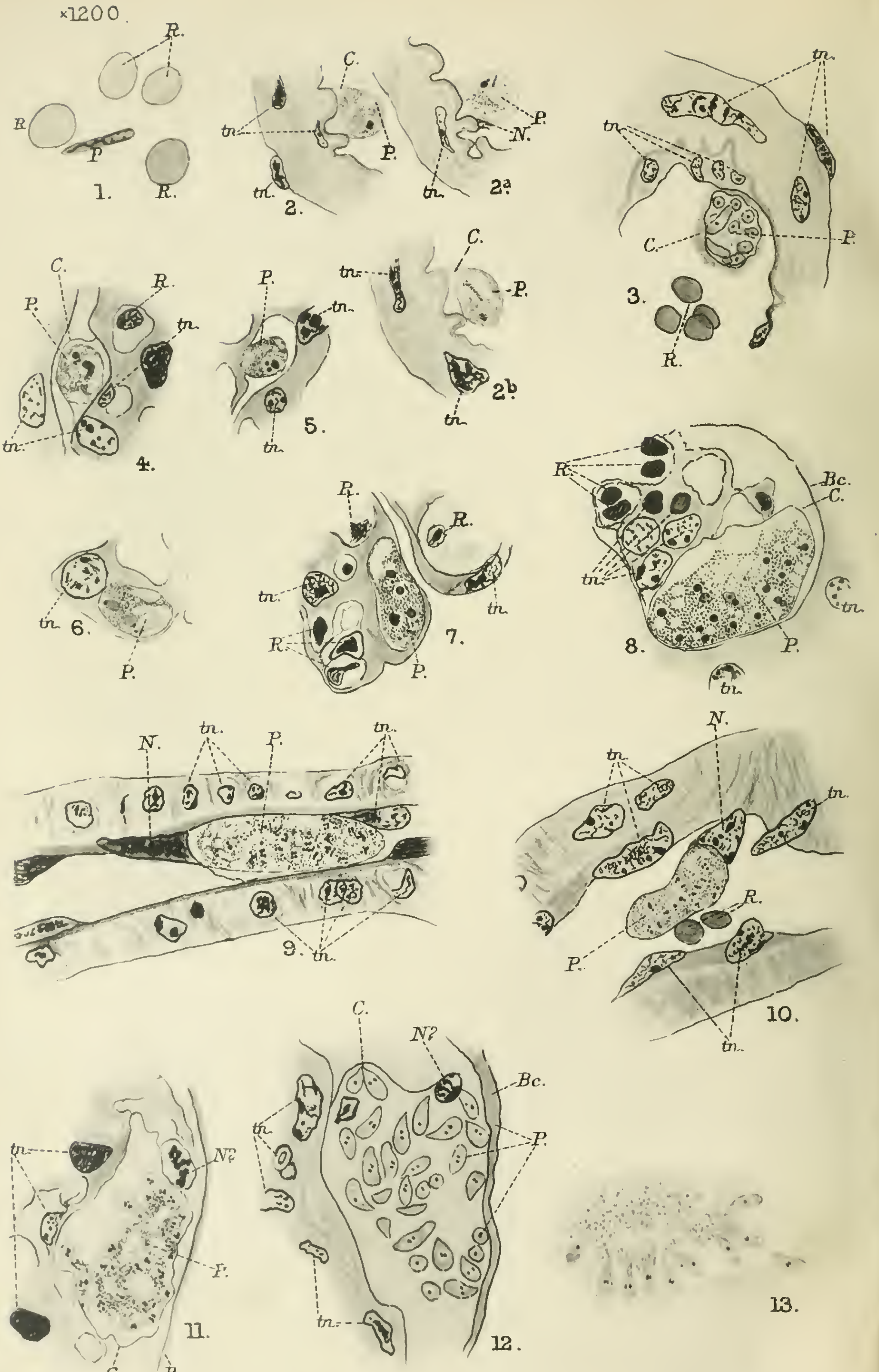
Figs. 32, 33.—Complete separation into sporoblasts. Increase of nuclei.

Figs. 34, 35.—Further increase of nuclei. In fig. 35 arrangement of nuclei about the equator of a sporoblast.

Figs. 36, 37, 38.—Sections through three spores showing sporozoites.

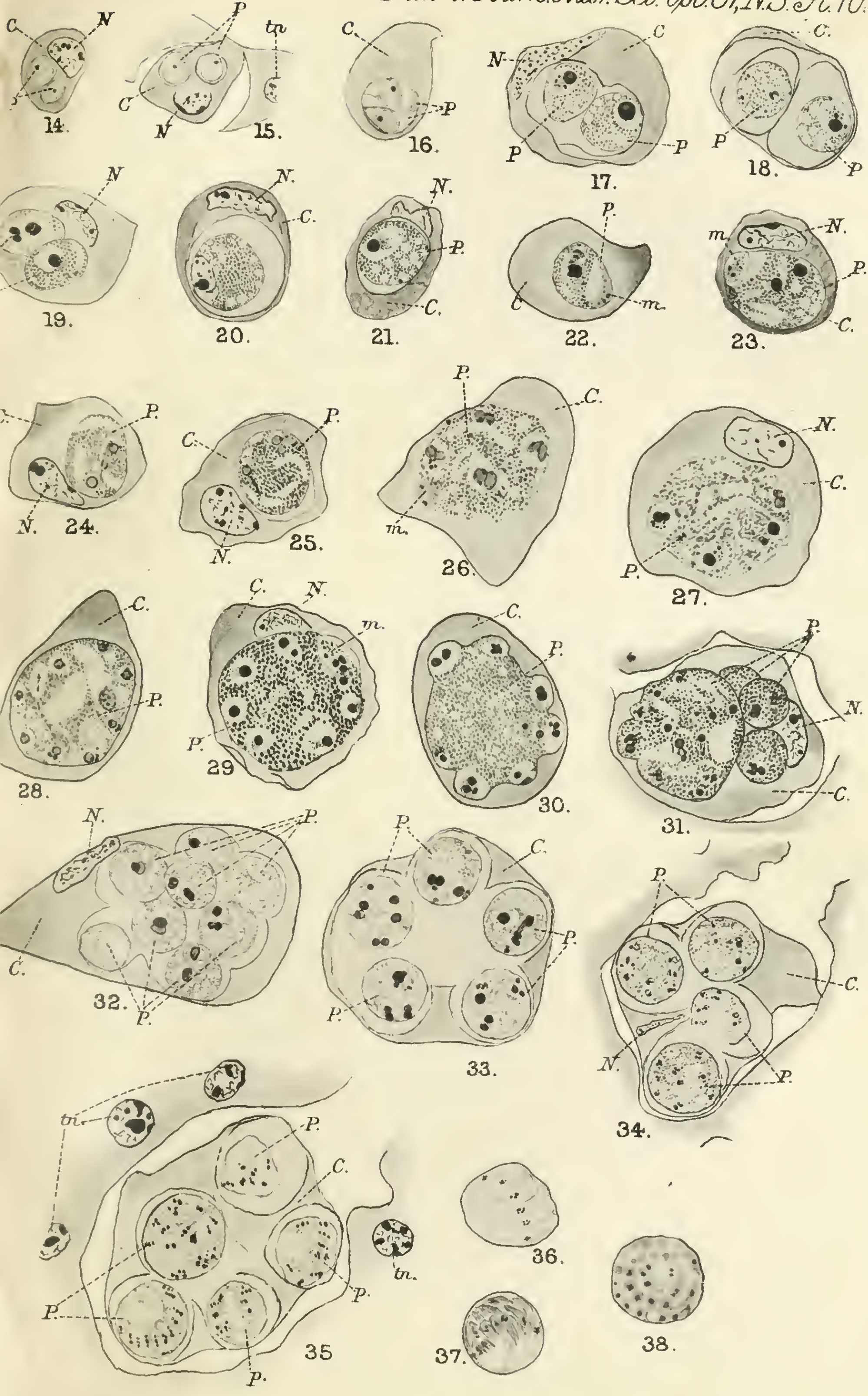
1380

[illegible] No 10



STEVENSON — KL

Quart. Journ. Micr. Sci. Vol. 61, N.S. Pl. 10.



ELLA MURIS.

Huth, coll.

London University)

UNIVERSITY COLLEGE HOSPITAL
MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

RESEARCH DEPARTMENT

Director :
Prof. A. E. BOYCOTT, F.R.S.

COLLECTED PAPERS

VOL. VI.—JUNE 1920.

CONTENTS.

1. The effect of division of the hypogastric nerves on frequency of micturition. By F. J. F. Barrington. *Quarterly Journal of Experimental Physiology*, vol. ix., (1915), p. 261. – 264

2. Observation on the pathology of cardiac dropsy. By C. Bolton. *British Medical Journal*, May 19th—26th 1917.

3. Further observations on the pathology of cardiac dropsy. By C. Bolton. *Journal of Pathology and Bacteriology*, vol. xx., (1919) p. 290.

4. Pathological changes in the adrenal glands. By T. R. Elliott. *Quarterly Journal of Medicine*, vol. viii., (1914), p. 47,

5. Further remarks on the ventricular extrasystoles and fibrillation under chloroform. By A. G. Levy. *Heart*, vol. vii., (1919), p. 105. – 110.

6. The spread of the excitatory process in the vertibrate heart. By T. Lewis. *Philosophical Transactions, B*, vol. ccvii., (1916), p. 221. – 310

7. The nature of serum antitrypsin and its relation to autolysis and the formation of toxins in infection and anaphylaxis. By F. H. Teale and E. Bach. *Proceedings of the Royal Society of Medicine*, vol. xiii., (1920), *Section of Pathology*, p. 5.

8. The relation of the antitryptic titre of the blood to bacterial infection and anaphylaxis, By F. H. Teale and E. Bach. *Proceedings of the Royal Society of Medicine*, vol. xiii., (1920), *Section of Pathology*, ~~p. 43.~~

9. The paths of spread of bacterial exotoxins with special reference to tetanus toxin. By F. H. Teale and D. Embleton. *Journal of Pathology and Bacterjology*, vol. xxiii., (1919), p. 50.

10. Paroxysmal heart-block with paroxysmal auricular fibrillation. By K. D. Wilkinson and H. G. Butterfield. *Heart*, vol. vi., (1915), p. 3.

11 261-264

THE EFFECT OF DIVISION OF THE HYPOGASTRIC NERVES ON FREQUENCY OF MICTURITION.[1] By F. J. F. Barrington. (From the Graham Research Laboratories, University College Hospital Medical School.)

(*Received for publication* 11*th October* 1915.)

In a previous paper[2] two experiments were described which bore on this point, but their results were not very conclusive. Five further experiments have since been performed in the same way. Briefly, the method consists in keeping the animal (cat) in a cage placed on a metal tray which slopes towards the centre, where there is a hole: urine runs through the hole into a receiver placed below: the receiver is visited periodically and the quantity of urine passed and the number of micturitions noted.

Exp. 1. Female cat which had been in the laboratory about a week when the experiment began on 29th December 1913:—

Week.	Number of micturitions.	Total quantity of urine passed in c.c.	Mean quantity of urine passed at each micturition in c.c.
1st . . .	14	715	51
2nd . . .	9	510	57
3rd . . .	9	575	68
4th . . .	12	530	44
5th . .	12	535	45
6th . . .	10	490	49
7th . .	11	580	53
8th . . .	12	590	49
Inferior mesenteric ganglia excised.			
9th . . .	17	655	39
10th . .	18	560	31
11th . .	19	565	30
12th . .	21	520	25
13th . . .	21	440	21
14th . .	25	375	15
15th . . .	30	425	14

---

[1] The expenses of this research were defrayed by grants from the Royal Society and the Graham Research Fund.

[2] Quart. Journ. of Exper. Physiol., vol. viii. p. 33.

Exp. 2. Female cat which had been in the laboratory a few days when the experiment began on 23rd March 1914:—

Week.	Number of micturitions.	Total quantity of urine passed in c.c.	Mean quantity of urine passed at each micturition in c.c.
1st . . .	8	690	86
2nd . .	9	545	61
3rd . . .	11	425	39
4th . . .	12	500	42
5th . . .	9	570	63
Inferior mesenteric ganglia excised.			
6th . . .	9	455	51
7th . . .	8	420	52½
8th . . .	10	480	48
16th . . .	13	485	37
17th . . .	12	435	36

Exp. 3. Male cat which had been in the laboratory about three weeks when the experiment began on 29th June 1914:—

Week.	Number of micturitions.	Total quantity of urine passed in c.c.	Mean quantity of urine passed at each micturition in c.c.
1st . . .	7	495	71
2nd . .	7	480	69
3rd . . .	6	480	80
4th . . .	9	565	63
5th . . .	8	450	56
Inferior mesenteric ganglia excised.			
6th . .	...	...	...
7th . .	11	560	51
8th . . .	14	720	51
9th . . .	12	645	54
10th . .	13	650	50
11th . . .	17	615	36
12th . . .	18	565	31

Exp. 4. Female cat which had been in the laboratory about three weeks when the experiment began on 29th June 1914:—

Week.	Number of micturitions.	Total quantity of urine passed in c.c.	Mean quantity of urine passed at each micturition in c.c.
1st . . .	6	515	86
2nd . .	7	595	85
3rd . . .	9	685	76
4th . . .	6	585	97
5th . . .	12	585	49
6th . . .	...	...	...
7th . . .	10	530	53
8th . . .	10	600	60
	Inferior mesenteric ganglia excised.		
9th . . .	7	390	56
10th . . .	17	525	32
11th . . .	13	485	37
12th . . .	11	450	42
13th . . .	16	520	32½
14th . . .	11	460	42

Exp. 5. Castrated male cat which had been in the laboratory about three weeks when the experiment was begun on 29th June 1914:—

Week.	Number of micturitions.	Total quantity of urine passed in c.c.	Mean quantity of urine passed at each micturition in c.c.
1st . . .	4	355	89
2nd . . .	8	675	84
3rd . . .	4	400	100
4th . . .	5	490	98
5th . . .	6	525	87½
	Inferior mesenteric ganglia excised.		
6th . . .	...	...	...
7th . .	12	610	51
8th . . .	13	575	44
9th . .	12	525	44
10th . . .	14	670	48
11th . .	10	545	54½
12th . . .	9	485	54

From these five experiments it appears that division of the hypogastrics in the cat is constantly followed by frequency of micturition. The object of excising the ganglia instead of merely dividing the nerves was, of course, to prevent regeneration in the period covered by the experiments. It is further seen that the frequency does not attain its maximum at once, but continues to increase for some weeks: this seems to indicate that after section of the hypogastrics no compensatory diminution in the motor tone of the pelvic nerves takes place, but the latter continues to overact, at any rate during the period of the experiment. No abnormality of micturition, other than frequency, was seen in these experiments except in No. 3. Twenty days after excision of the inferior mesenteric ganglia this cat was twice seen to micturate twice within a minute, passing 10 c.c. and 15 c.c. respectively; again eight weeks after the operation it was seen to pass 10 c.c., and the bladder on palpation immediately after was not empty but appeared to contain 20 to 30 c.c. This is the only cat in which residual urine was known to be present after division of the hypogastrics, and the only two occasions on which it was found in this experiment.

Some observations made on a rabbit appear to show that in this animal there are normally other factors in frequency of micturition than distension of the bladder. As is well known, rabbits can be kept in perfect health without giving them water to drink if they are fed properly; they will, however, frequently drink water if it is given them. In the following experiment observations were made in the same way as in the case of the cats. The rabbit, a male, was given no water during the first five weeks except on two days in the second week, by accident. From the sixth week onwards it was given water twice daily. At first it always drank freely, but later took less. The following table shows the results:—

Week.	Number of micturitions.	Total quantity of urine passed in c.c.	Mean quantity of urine passed at each micturition in c.c.
1st . . .	14	230	16
2nd . . .	12	435	36
3rd . . .	9	145	16
4th . . .	9	150	17
5th . . .	7	105	15
6th . .	7	405	58
7th . . .	11	525	48
8th . . .	11	470	43
9th . .	12	395	33
10th . . .	11	205	19
11th . . .	14	265	19
12th . . .	15	350	23

In this experiment, therefore, though there was a polyuria of nearly four times the original amount, there was at first no increase in the number of micturitions at all. This is the only experiment of the kind which I have successfully performed, as rabbits are not suitable for the method employed owing to the small amount of urine usually passed at each micturition. Attempts to produce polyuria in cats by decreasing the amount of meat given them, and giving large quantities of milk, always produced diarrhœa, which vitiated the result of the experiment.

Observations
ON
THE PATHOLOGY OF CARDIAC DROPSY.

LECTURE I.

[After expressing his sense of the honour conferred upon him by the College, Dr. Bolton referred to the work of Lower, Cohnheim, and Starling, explained that the object of his researches was to produce experimentally uncompensated heart disease in order to study the resulting pathological processes, and proceeded as follows:]

Interference with the Filling of the Heart.

Experiment had shown that the heart, with its muscle in all respects intact, can so perfectly accommodate itself to increased work, that it will overcome an increasing resistance opposed to it up to a certain point, and thus maintain the arterial and venous pressures at their normal heights; beyond this point, however, when the heart cannot completely overcome the resistance, it suddenly fails and the circulation comes to an end. One cannot, therefore, experimentally interfere with the heart in its capacity as a force-pump so as to produce a gradual heart failure.

The alternative method of preventing the free diastolic filling of the heart proved successful. The means which I adopted to interfere with this function consists in constricting the pericardium by means of a row of sutures placed in the right side of that structure. This proceeding exercises a certain amount of pressure on the right auricle and roots of the great veins, and interferes with the free expansion of the ventricles. The animals, which in all the experiments to be described were cats, were anaesthetized with ether, and artificial respiration was maintained through an intubation tube during the operation, which was performed under strict antiseptic precautions. On the following day the animal has become dropsical, and this condition gradually increases in degree. The maximum amount of venous engorgement is found towards the centre of the body, the superior and inferior venae cavae and the liver, whilst the periphery appears practically unaffected. The distribution of the dropsy corresponds with that of the venous engorgement; there is ascites and double hydrothorax with marked oedema of the mediastinum, and to a less extent of the retroperitoneal tissue. This oedema may spread along the great veins, more particularly the superior vena cava, so that it extends out of the chest, giving rise to subcutaneous oedema of the front of the neck and chest, and even of the axillae. This distribution of the subcutaneous oedema is evidently influenced by gravity.

The immediate effects upon the circulation of the blood, as measured by arterial and venous manometers, I found to be the same as in Cohnheim's experiment. The arterial pressure at once fell to varying degrees in proportion to the tightness of constriction, and the venous pressure in all parts of the body, head, feet, and portal vein, at once rose also to varying degrees. On observing the experiment for an hour or more I was, however, surprised to find that the venous pressure in all parts of the body gradually fell until it returned to the normal level, whilst the arterial pressure still remained at the low level to which it had fallen. On the following day, when dropsy was present, I was again surprised to find that the venous pressures were still normal or a little raised, and that the arterial pressure had now usually regained its normal height.

When I made these observations we had no idea what the arterial pressure was in cardiac dropsy, but three years later H. J. Starling published a series of clinical observations in which he showed that in uncompensated heart disease with dropsy the arterial pressure was normal, except in cases associated with arterial or renal disease when it was raised. The distribution of the subcutaneous oedema in the dependent parts of the body in cardiac dropsy is due to the upright position of the patient, so that the venous engorgement of the liver and veins of the central parts of the trunk is relieved by gravity at the expense of the legs. When it is necessary to tap the subcutaneous tissues, it is a common practice to let the patient sit up in a chair so as to drain the fluid into the legs. The changes are essentially the same as in these animal experiments, and the differences are entirely due to differences of position.

I concluded that the venous pressure fell owing to dilatation of the large veins of the trunk and of the liver, so that blood accumulated in the central parts, whilst it was abstracted from the periphery. In support of this supposition was the visible distension of these veins and the liver, and also the fact that, when the venous pressure had fallen to the normal level after its initial rise, relaxation of the pericardium brought about a further fall below the normal. The dilatation was due to the raised venous pressure and to a nutritional loss of tone in the vessels of the congested area. The dropsy was produced in this central area of the body whilst the capillary pressure was about the normal, and therefore I concluded that the oedema was primarily due to some alteration in the capillary wall.

From these observations it was quite evident that certain adaptive changes had occurred which were worthy of closer study, but the method of pericardial constriction was not a satisfactory one to employ for a large series of experiments, because so many animals died from the operation, as it was quite impossible to gauge the amount of constriction.

Obstruction of Both Venae Cavae.

I therefore employed another method for producing the same result. This consisted of simultaneous obstruction of the superior and inferior venae cavae. The constrictions were applied immediately above and below the right auricle, the azygos vein being ligatured. The average diameters of the veins were obtained by washing out the blood and distending them with gelatine at the normal venous pressure. After the gelatine had set the vessels were cut out and measured. The veins were constricted to varying degrees by tying round them short pieces of split rubber catheter of known diameters. I found that constriction of both veins led to a condition of dropsy precisely similar in its distribution to that produced by pericardial constriction, and that the effects upon the venous and arterial pressures were also the same.

Obstruction of Inferior Cava Alone.

An incomplete obstruction of the inferior vena cava alone produces no effect whatever unless the vein is narrowed down to three-fifths of its normal diameter; the animal survives, and develops dropsy. Obstructions greater than this produce similar results, but narrowing down below two-fifths of the diameter of the vein kills the animal as in complete ligature. One is thus able to guarantee the survival of any animal by using a rubber tube of 2.5 to 3 mm. in diameter, and also that the animal will develop dropsy. The ascites is rapidly produced, measurable amounts being present after two hours, and large amounts during the first few days; it then slowly increases up to the third or fifth week, as a rule, but sometimes for a longer period; it then slowly subsides with the establishment of anastomoses, and has disappeared in from two to six months. Now and then it may persist longer than six months. In addition to ascites there is commonly a little oedema round the inferior vena cava behind the peritoneum, and exceptionally it may extend from this locality down the iliac veins to the groin; here it forms a bag of fluid, which further extends along the anterior abdominal subcutaneous tissue, and down the thigh to the knee. This extension is due to gravity, the position assumed by the animal favouring this distribution of the subcutaneous fluid.

The appearance of oedema at a certain point in the subcutaneous tissue does not necessarily imply that it is formed in this position, because fluid is able to diffuse itself in the areolar tissues much in the same way as subcutaneous emphysema; in this form of diffusion gravity plays an important part.

Consequent Pressure Changes.

We have now to consider the pressure changes in the various parts of the circulatory system as a result of this obstruction of the inferior vena cava.

Immediate Changes.

A gradually increasing constriction produces no effect whatever on the pressures until the vein has been narrowed to three-fifths of its diameter, when the pressure at the lower end of the inferior cava rises by about 30 or 40 mm. $MgSO_4$ solution. The normal pressure in this situation is about 90 to 100 mm. $MgSO_4$, so that the rise is about one-third of the normal. The solution was made of a specific gravity of 1046, so that it is one-thirteenth the weight of mercury. A rise of 30 to 40 mm. therefore represents about 2 to 3 mm. Hg. At exactly the same point the arterial pressure falls by 20 mm. Hg or more owing to deficient filling of the heart. Constrictions greater than 3 mm. produce somewhat greater depressions in the arterial pressure curve and greater rises in the venous, and complete ligature of the vein causes the arterial pressure to sink to about 30 mm. Hg above zero, whilst the venous pressure behind the obstruction runs up rapidly to over 200 mm. $MgSO_4$. To cause a high degree of venous pressure, therefore, the vein must be completely occluded. The rise of venous pressure is not limited to any portion of the venous area drained by the inferior vena cava, but extends back to the venous radicles at all points. Thus a cannula placed in a vein of the foot shows precisely the same increase of pressure in the manometer, and the rise of pressure is also propagated through the liver into the splenic vein to the same degree. On the other hand, the pressure in the superior vena cava and its tributaries falls, the fall amounting to about 10 or 15 mm. $MgSO_4$ solution.

We see, thus, that there is a redistribution of the amounts of blood in the different parts of the body; by its accumulation in the inferior vena cava and its tributaries it has been abstracted from the rest of the venous system and the arteries. The capillary pressure in all the parts drained by the inferior vena cava rises, whilst the velocity of the blood is diminished. This rise we have seen to be on the average about 2 to 3 mm. Hg, so that, if the normal capillary pressure were 30 mm. Hg, after obstruction of the vein the pressure would stand at 32 to 33 mm. Hg. This is a comparatively insignificant rise. We must, however, remember that the height of the capillary pressure depends not only upon the rate at which blood is leaving the capillaries, but also upon the rate at which it is being supplied to them. If the arterial pressure remained normal and these vessels contained the normal amount of blood, the capillary pressure after obstruction would stand at the height indicated above, but blood is abstracted from the arteries and the pressure in them falls; less blood than normal is therefore supplied to the capillaries, so that for this reason the pressure within the latter is not raised to the level indicated by the height of the venous pressure. Exactly what lessening of effect is produced by the fall of arterial pressure it is impossible to say; we can only conclude that the capillary pressure is raised, but to a smaller extent than one would expect from the height of the venous pressure, and in any case the rise is trifling. The fall of venous pressure in the superior vena cava and its tributaries, together with the fall of general arterial blood pressure, naturally results in a fall of capillary pressure in these parts.

These are the immediate effects of narrowing of the inferior cava, and they do not last for very long, because the venous pressures in all the parts of the body behind the obstruction commence to fall gradually, and, if the animal be kept under observation for an hour or more, it will be found that the venous pressures in the inferior vena cava itself, in the portal vein, and in the foot eventually reach the normal level or a point very near it. The venous pressure in the head, however, at the end of this time still remains at the low level it had immediately assumed on obstruction of the vein. The arterial pressure in all cases remains at the level to which it had fallen on the occurrence of obstruction, or it may be lower still. The higher the pressure in the inferior cava the quicker it falls to the normal level.

This fall of venous pressure is due to precisely the same cause as we have seen to be operative in constriction of the pericardium—namely, dilatation of the veins and capillaries of the liver more especially, but also of the portal system, and of the inferior vena cava and its immediate tributaries.

This distensibility of the abdominal vessels is responsible for a further depletion of the fore part of the body, and the hind limbs and peripheral portions of the abdomen now share in this depletion. Owing to the increased capacity of a part of the circulatory system, the pressures in all parts of the system naturally fall, the blood volume remaining normal. The venous pressures in all parts behind the obstruction being normal or thereabouts, and the arterial pressure low, it naturally follows that the capillary pressure is subnormal in these parts, except in the liver, where it is about normal. The capillary pressure in the fore part of the body falls lower than it was before.

This is the condition of the circulation when dropsy commences; an increased output of lymph occurs in the plethoric areas giving rise to ascites and some degree of retroperitoneal oedema. The ascitic fluid comes from the viscera, especially the liver, and the oedema from the capillaries of the short lumbar veins directly opening into the inferior cava. The veins of the abdominal wall and the hind limbs actually contain less blood than normal. Increased absorption of fluid occurs in these regions and also in the fore part of the body owing to the deficient filling of the vessels and the resulting low capillary pressure.

The output of lymph in the plethoric areas is thus balanced by the absorption of fluid in these depleted areas, the blood volume remaining normal. It is well known that such an absorption always occurs, because Lazarus-Barlow has shown that, when a part is rendered plethoric and oedematous, the specific gravity of the tissues in the anaemic areas of the body rises, showing that fluid has been absorbed from the tissues into the blood.

The capillary pressure being normal or thereabouts, the increased output of lymph is entirely conditioned by the state of the vessel wall. Exactly what this state is one can merely surmise, but it can hardly be doubted that the normal nutritional exchanges of a part in which there is such a high degree of stasis of blood are seriously interfered with. So far as we can see at present the fault is primarily a nutritional change in the capillary wall, and what part is played by filtration at the normal capillary pressure, or by osmosis resulting from altered tissue metabolism, must at the present moment be left an open question.

All the changes which I have hitherto described occur during the first few hours after the vein is obstructed, and we have now to consider what later alterations in the pressures take place. For this purpose a series of dropsical animals was examined at various intervals after the operation until the dropsy had disappeared.

Later Changes.

The later alterations occur in two stages, and they are due to two distinct causes:

1. The first stage is characterized by a rise of venous pressure, approximately to the height from which it had fallen, and a rise of arterial pressure to about the normal level.
2. During the second stage the venous pressure rises to a high degree, the pressures which I have recorded being comparable to those following complete ligature of the vein. The arterial pressure still remains at the normal level.

First Stage: Restoration of Venous and Arterial Pressure.

The alterations in the circulation at the first stage are usually completed in a few hours, nearly always within twenty-four. In one case on the fifth day, however, I have found a normal venous and subnormal arterial pressure, although the animal had 100 c.cm. of fluid in its abdomen. At this stage the venous pressures in the portal vein and inferior vena cava gradually rise until they attain the heights at which they stood immediately after the operation. The pressure, however, in the veins of the legs always remains relatively lower than that in the central part of the trunk. This pressure does not rise above the normal, and unless the pressure in the trunk is very definitely raised it is subnormal. There is, therefore, a relative alteration in these two pressures; the venous pressure in the trunk is always relatively raised, and that in the legs relatively lowered. The arterial pressure rises to the normal, varying somewhat with the venous pressures in the trunk. The venous pressure in the territory of the superior vena cava still remains at the low level it immediately assumed when the operation was performed. This again shows, as we saw before, a relative plethora of the central parts of the trunk, and depletion of the peripheral parts of the circulation.

The Cause of this Alteration in the Venous and Arterial Pressures.

In the normal animal the arterial pressure is kept at a constant level in order that the capillaries may be supplied with an adequate flow of blood. This especially applies to the brain, so that the medulla may receive its normal supply of oxygen. Whenever the arterial pressure falls, so that the vasomotor centre does not receive its proper supply of blood, it is excited to action and the blood pressure rises. One would expect, therefore, that the low arterial pressure in the dropsical animals would excite the vasomotor centre to action so as to produce a general arterial constriction, with its resulting rise of arterial pressure and a greater flow of blood to the brain. It might, however, be argued that in these cases absorption of fluid had overbalanced output and brought about hydraemic plethora, and that this increase in the total blood volume was the cause of the raised vascular pressures. I have, however, shown that at this stage of the disease there is no increase in the blood volume. It must therefore be by vasomotor constriction, which automatically occurs by excitation of the vasomotor centre as the result of anaemia of the medulla, that the arterial pressure becomes raised to the normal. Naturally, this result varies in different animals, according to their power of recovery after a severe operation. In some cases it rapidly occurs, so that the arterial pressure rises to its originally normal level in three or four hours, whilst in others the pressure still remains low on the following day or even later. It was shown by Bayliss and Starling that stimulation of the splanchnic nerves not only raises arterial blood pressure, but also the pressure in the inferior vena cava by 20 to 30 mm. $MgSO_4$ solution.

Vascular constriction, therefore, fully accounts for the rise of arterial and venous pressures. It is an adaptive mechanism enabling the animal to compensate as far as possible for its changed circulatory conditions. The rise of arterial pressure ensures a normal supply of blood to the brain, and the raised venous pressure increases the velocity of blood through the constriction, so that the maximum amount possible may reach the heart and be distributed to the tissues.

These alterations in venous and arterial pressures in their turn affect the capillary pressure. The rise of venous pressure results in a rise of capillary pressure in the central parts of the trunk, but this rise is minimized by the arterial constriction which cuts down the supply of blood to the capillaries, so that it is doubtful whether the capillary pressure rises above its normal level except in the liver, where it must go up, because both portal and inferior cava pressures are raised. The capillary pressure in the legs, abdominal wall, and fore part of the body is subnormal, and increased absorption continues in these regions as before. The alteration in pressures does not obviously affect the output of lymph, because there is no distinct relation between the height of the venous pressure in the trunk and the amount of ascites present.

Second Stage: Rise of Venous Pressures to a Great Height.

In these animals the venous pressures are very high after the first week and occasionally during this period, but it is not until the end of the second week that they attain their maximum heights. The maximum pressures occurred from the second to about the eighth week. The pressure in the portal vein followed that of the inferior cava, and was raised to about the same proportionate extent. The venous pressure in the hind limbs was always raised above the normal after the second week, but to a less relative extent than that of the inferior cava. Although all the venous pressures have gone up, there is still the same alteration in the pressure levels as described before, the venous pressure in the trunk being relatively more raised than that in the hind limbs. This occurrence is, therefore, a constant feature at all stages of the disease. Another constant feature refers to the pressure in the superior vena cava, which in all cases was still below its normal level, as immediately after the vein was constricted. The same disproportion in the distribution of the blood as we saw to occur in the early stages is, therefore, still present. There is a relative plethora of the central portion of the trunk, and relative anaemia in the peripheral parts. The arterial pressure was normal, although in four cases it was a trifle higher than usual. The capillary pressures are raised in all parts behind the obstruction, but to a less relative extent in the hind limbs than in the trunk; those of the fore part of the body are below normal.

With regard to the influence of this high venous pressure upon the production of dropsy, it may be definitely stated that there is no constant relation between the height of the venous pressure and the amount of ascitic fluid present, and that, moreover, during the subsidence of the ascites and after it has disappeared the venous pressure still remains high. These observations upon the venous pressures in the later stages of the disease, therefore, agree with those made in the earlier stages in showing that a high capillary pressure is not the primary cause of the dropsy and is not necessary for its production. Neither is the presence of a high pressure capable of preventing the subsidence of the dropsy. It is true that the period of highest pressure corresponds with some increase in the ascitic fluid, although an examination of individual cases does not bring out this point, because the ascites begins to subside at different times in different animals whilst the pressure is still high. So that when once oedema is being produced a high capillary pressure will increase it, although the two conditions may be dependent upon some other factor.

It seems quite clear that some new factor must have been introduced to cause this additional rise of venous pressure, which in half the cases becomes more than double its original height. Such a factor can only be an increase in the total quantity of the blood, and this leads us to inquire whether absorption in the anaemic parts of the body may not have eventually overbalanced output in the plethoric parts and led to a condition of general plethora; and, further, if the total blood quantum is increased, what is the nature of the increase, and what is its effect upon the production of lymph?

Establishment of Plethora.

That plethora occurred in certain forms of dropsy had been suggested by clinicians for many years before Cohnheim definitely opposed the idea. He considered it as proved that a lasting pathological increase in the blood quantum could not occur, because it had been shown that, when the blood volume of the dog was increased by transfusion of large amounts of blood, the excess was disposed of in a relatively short space of time.

I am aware of one case only in which the blood volume of a patient with uncompensated heart disease was actually estimated. This was an observation of Lorrain Smith, in which he found an increase in the blood volume. I have estimated the blood volume of twenty-five dropsical cats at all stages of the disease. The volume of the blood was obtained by the washing out method. The blood volume of a cat weighing 2,500 grams is about 100 c.cm. Ten estimations were made on cats during the first week. In five cases the blood volume was normal, in three it was diminished by 6, 9, and 16 c.cm., in two it was increased by 4 and 7 c.cm., so that during the first week the blood volume is normal or decreased; an increase may occur at this early date, but it is very slight and unable to affect the venous pressure. This period, it will be remembered, is that during which the venous pressure is raised by vaso-constriction alone. Four estimations were made during the second week. The blood volume of the animal on the eighth day was diminished by 10 c.cm. and the other three increased by 6, 15, and 28 c.cm. Towards the end of the second week, it will be remembered, the venous pressures tend to rise and afterwards remain uniformly high. Between the second and eighteenth week eleven estimations were made, and all showed an increase. The increases at this period were on the whole much greater than those of the second week. The lowest figures were from 7 to 18 and the highest from 23 to

47 c.cm. Considerable increases in the blood volume thus occurred during the period when the venous pressures were uniformly very high. It seems justifiable to conclude that about the second week absorption of fluid overbalances output and leads to an increase in the total blood volume. This causes a rise of venous pressure in the parts of the body behind the obstruction, but the quantity of blood in the previously anaemic area is not made up to the normal, and the pressure in this region is subnormal. Absorption, therefore, continues and keeps the blood volume above the normal. This implies a fairly rapid circulation of fluid into and out of the blood, and that this actually happens we shall see later in speaking of the extravascular circulation of the lymph.

This rapid and constant intake of fluid, therefore, seems to be the actual cause of the plethora, otherwise, as in Cohnheim's experiment, the excess of blood would be rapidly disposed of and the blood volume return to the normal. The condition of plethora in these experiments is not analogous to the artificial production of plethora by injection of fluid, because its maintenance depends upon a constant intake of fluid. Fluid is also rapidly leaving the vessels as ascites, but, as I shall show later, absorption of the ascites is constantly occurring into the blood so that by this rapid and constant circulation of fluid the volume of blood is permanently increased. During the first two weeks the blood volume bears no relation to the amount of ascitic fluid. The formation of ascites is very active from the first day and the fluid accumulates in large quantities during the first week. During this period the volume of blood is usually normal or even diminished, and as a fact the amount of ascites is commonly large when the blood volume is small. The increase in blood volume can in no way, therefore, be said to be the cause of the dropsy, because the essential cause or causes are at work before the blood increases in volume. The increase in blood volume must, therefore, be looked upon as a secondary phenomenon and due to an effort of the animal to make up its normal quantity of blood in the anaemic areas, and to replace the fluid lost as dropsy. At the same time it may be a contributing factor and assist in increasing an already existing dropsy. This appears to be the case, for the dropsy tends to increase about the time the volume of the blood increases, and after the second week is passed the amount of ascitic fluid varies more or less directly with the blood volume. However this may be, after the fifth week, when the dropsy is subsiding, the blood volume still keeps high. So that an increase in the blood quantum is unable to prevent the disappearance of the dropsy.

A further effect of this increase in the volume of the blood and the resulting high venous pressure is seen in the liver. In the early stages we have seen that the pressure in it is very little above the normal, and the capillaries are dilated moderately; in the stage of plethora, however, the pressure in this organ is considerably raised, the capillaries are dilated into sinuses, and the liver tissue atrophies.

It is obvious that if the total amount of fluid in the body is increased water must have been retained. The sources from which the blood derives fluid in order to increase its volume are the alimentary canal and the extravascular tissues. The daily volume of urine is diminished to about one half during the first week, after which it becomes normal, with a tendency towards an increase in the later stages. Less fluid is, however, consumed during the first few days, and, as a fact, the amount of fluid taken in and that excreted run parallel throughout the disease. Taking into account all sources of error involved in the method employed, it appears that there is retention of fluid in the body during the early period of oedema formation and increase of the blood volume, and a slight loss of fluid during the subsidence of the dropsy. That the excretory capacity of the kidneys in these animals is not materially interfered with is proved by the readiness with which they respond to an increase in the available amount of fluid presented to them to excrete. The retention of water appears, therefore, to depend primarily upon the circulatory processes at work, which are causing the dropsy.

The fluid which is absorbed directly into the capillaries from the extravascular spaces, so far as is known at present, consists of water and salts only. There are no direct observations to prove that protein is absorbed by the capillary wall.

Changes in Haemoglobin Content.

During the past twenty years or more the condition of the blood in uncompensated heart disease as regards its content of water has been investigated from time to time. The methods employed have been either that of ascertaining the total solids of the blood, of measuring its specific gravity, or of ascertaining the percentages of haemoglobin and red corpuscles which it contained. Many of these clinical observations agree that in cardiac dropsy hydraemia is present, but others differ from them in finding the haemoglobin content of the blood normal or even increased.

I have made 182 haemoglobin observations on 52 dropsical cats, and have estimated the total solids of the blood, serum, lymph, and ascitic fluid of 11 cats at various stages of the disease. These observations have shown that on some day during the first week the percentage of haemoglobin in the blood begins to decrease. This never occurs on the first day after the operation, every case examined at the end of twenty-four hours showing a normal or slightly increased percentage. It may commence on the second day, but only in about one out of six cases. Two-thirds of the cases show a commencing fall of the haemoglobin percentage on the third day, and the remainder on the fourth, fifth, and sixth days. One case showed a normal percentage on the eighth day. With this last exception every case out of seventy-six estimations, conducted from the seventh day to the end of the fifth week, showed a decrease in the haemoglobin percentage.

After this period, although the blood volume remains increased, the haemoglobin percentage rises, becoming normal or slightly above this level in most of the cases during the next few weeks.

On comparing the haemoglobin percentage with the blood volume it appears that the period of continuous increase in the blood volume corresponds with the period of continuous decrease in the haemoglobin percentage, and that after this, although the blood volume remains higher than normal, the haemoglobin percentage commences to rise. It is therefore evident that the decrease in the haemoglobin content of the blood is due to a dilution of the blood, and not to a decrease in the total amount of haemoglobin. The rise in haemoglobin percentage whilst the blood is increased in volume represents an effort on the part of the animal to restore this percentage to the normal. This it succeeds in doing sometimes whilst the ascites is present, and sometimes after it has disappeared.

Here is to be found the explanation of the discrepancies which have occurred in clinical observations on the haemoglobin content of the blood. The alterations in the haemoglobin percentage and the corpuscular content of the blood pass through very definite stages, so that a normal, an increased, or a diminished percentage in either of these is found at different times in the same individual. The formation of new blood corpuscles is probably due to the diminished oxygen tension of the blood. This is brought about in uncompensated heart disease by the diminished velocity of the blood and the cyanosis.

Changes in Blood Plasma.

We have now to discuss the changes which occur in the blood plasma. The total solids in the ascitic fluid amount to about 5 or 6 per cent., and occasionally 7, being slightly less than those of the lymph in any given animal. A considerable amount of protein is thus rapidly lost by the blood, the diminution in volume of the latter being made up by the absorption of water. There results, therefore, in the earliest stages a simple hydraemia, in which the total volume of the blood is normal, but the percentage of water in the plasma is increased. The total solids of the blood and serum are diminished, but the haemoglobin percentage is normal, showing that the total blood volume is normal.

We know very little about the formation of the blood proteins beyond the fact that they are formed fairly rapidly. My observations on the total solids of the blood and the serum fully bear out this point, because I have found that after the first day, whilst the total solids of the blood are uniformly and considerably diminished, together with the percentage of haemoglobin, the solids of the serum are only a little less than normal. This means that the animal rapidly makes up the proteins of its plasma, so that the preliminary hydraemia tends to diminish. After the fifth week has passed, and the excessive lymph production is falling off, the total solids of the serum become absolutely normal.

We must therefore distinguish several phases through which the blood passes: A simple hydraemia, the total volume of the blood being normal or even diminished; a plethora in which the plasma is to some extent hydraemic and the percentage of corpuscles diminished; a true plethora in which the plasma is normal in composition and the percentage of corpuscles has also reached the normal. The proteins of the blood are thus made up much more rapidly than the corpuscles, so that one cannot say that hydraemic plethora exists merely because the corpuscles are diminished in percentage. Were it not for the constant absorption of water and output of protein, tending to keep the blood hydraemic, the proteins of the blood would, no doubt, be found normal in quantity from an early date. We have already seen how the organism attempts to compensate for the anaemic condition of certain portions of its vascular system by vaso-constriction, whereby it ensures that the maximum amount of blood possible reaches the tissues. It has only a certain quantity of available blood at its disposal, and with this it is unable to supply the tissues with their normal amount. A further series of events is therefore initiated whereby the blood is first increased in quantity, and then becomes normal in quality.

SUMMARY.

1.

The increased output of lymph giving rise to cardiac dropsy is one of a very definite series of events following the occurrence of failure of the heart in its capacity as a pump. These events may be grouped into the following stages:

Stage I.—Accumulation of blood in the veins, with a moderate rise of venous and capillary pressure in all parts. Abstraction of blood from the arterial system with a fall of arterial blood pressure.

Stage II.—Dilatation of the veins and capillaries of the central parts of the body, with a fall of venous and capillary pressure to normal or thereabouts. This leads to increased congestion of the central parts and anaemia of the peripheral parts of the body, in which the venous and capillary pressures fall below normal. Increased output of lymph in the congested area giving rise to dropsy. Increased absorption of water in the anaemic areas, which balances the loss of fluid by the blood. Simple hydraemia results.

Stage III.—Vasomotor constriction resulting from anaemia of the medulla causing:

1. A rise of arterial pressure to the normal.
2. A moderate rise of venous and capillary pressure in the central parts of the trunk.

The venous and capillary pressures of the peripheral parts remain subnormal, or may become normal.

Stage IV.—Hydraemic plethora is established and raises the venous and capillary pressures in the central portions of the body to a great height. There is still a relative anaemia of the peripheral portions, although to an increasingly limited extent, as the congested area spreads peripherally.

Stage V.—The ascites and hydrothorax increase in degree and the oedema spreads in the areolar tissues peripherally, as the congested area encroaches upon the anaemic regions. True plethora is established.

2.

The abnormal output of lymph leading to dropsy is primarily conditioned by the nutritive state of the capillary wall, and is increased by the general plethora and consequent high capillary pressure.

3.

In uncompensated heart disease in man the central portions of the trunk are relieved by gravity at the expense of the legs, which become included in the congested area. The dropsical area spreads up the legs to the abdominal wall, thus encroaching upon the anaemic area from below upwards.

BIBLIOGRAPHY.

Bayliss and Starling: *Journ. Physiol.*, 1894, vol. xvi, p. 171.

Bolton: *Journ. Path. and Bact.*, 1903, vol. ix, p. 67. *Proc. Roy. Soc.*, London, 1907, B, vol. lxxix, p. 267. *Journ. Path. and Bact.*, 1909, vol. xiv, p. 49. Ibid., 1916, vol. xx, p. 290.

Cohnheim: *Lect. Gen. Path.*, New Syd. Soc. Trans., London, 1889, vol. i, pp. 21, 513, 423

Hammerschlag: *Zeit. f. klin. Med.*, Berlin, 1892, Bd. xxi, S. 475.

Lazarus-Barlow: *Phil. Trans.*, B, 1894, vol. clxxxv, p. 779.

Lorrain Smith: *Trans. Path. Soc.*, London, 1902, vol. liii, p. 136.

Lower: *Tract. de Corde*, etc. Edit. sex., London, 1728, p. 127.

Starling, E.: Arris and Gale Lectures, *Lancet*, 1896, vol. i, pp. 1267, 1407. Ibid., 1897, vol. i, pp. 569, 723.

Starling, H. J.: *Lancet*, 1906, vol. ii, p. 846.

LECTURE II.

We have now to consider the question of the abnormal exchange of fluid between the blood capillaries and the tissue spaces, which leads to an excess of tissue fluid in the congested area, to a deficiency in other parts, and to an increase in the total blood volume.

Absorption in Area of Superior Vena Cava.

It has been shown that when the inferior vena cava is obstructed the venous pressure in the fore part of the body falls, owing to abstraction of blood from this region, and also that at all stages of the disease this pressure is still below normal. The circulatory conditions in this part are, therefore, such as to determine a continuous absorption of fluid by the capillaries from the tissue spaces. It is not to be supposed that absorption is the only process occurring in this region, but that a new balance has been struck between lymph production and absorption. Exactly how this condition is brought about is not known, except that the low capillary pressure is a factor in the process of absorption.

Starling was the first observer to show that proteins are able to exert a definite though small osmotic pressure. In the case of the proteins of the blood plasma he found this pressure to be about 30 mm. Hg as compared with isotonic salt solution. The protein concentration of the extravascular fluid is a quarter or a third of that of the blood plasma, so that the latter is able to exert a definite osmotic attraction for the extravascular fluid. The force of this attraction, as I have already mentioned, is about equal to the hydrostatic pressure in the capillaries, so that when the latter falls below the normal level water will be absorbed.

Absorption in Area of Inferior Vena Cava.

Turning now to the consideration of the exchange of fluid in the area drained by the inferior vena cava our task is not so simple. I have so far assumed that increased absorption is occurring at the periphery as in the fore part of the body.

This question of absorption at the periphery can be examined experimentally, because the parietal peritoneum, which is included in this region, is particularly suitable for such experiments.

With regard to the visceral peritoneum, it is quite clear that output has overbalanced absorption, so that in order to find out what is happening in the case of the parietal peritoneum, we must inquire into the relative parts played by these two processes in the peritoneum generally during the various stages at which the fluid is accumulating, remaining stationary, or disappearing.

Output into Peritoneal Cavity.

Our inquiry into the subject must first be directed to the relative rates of output of fluid into the peritoneum at the different periods of the disease. I shall show later that absorption from the peritoneum is occurring always at the same rate, so that a comparison of the rates at which the ascites reaccumulates after the abdomen has been thoroughly emptied should give us the required information. For this purpose the animal was anaesthetized and the abdomen opened under aseptic precautions. The fluid was then removed with a graduated pipette and the abdomen closed. I found that, however large or small the amount of fluid removed, and whatever the stage of the disease, the ascitic fluid had reaccumulated on the next day.

It may be stated generally that the rate of output is rapid from the beginning, that it increases to some extent as the blood increases in volume, and that it diminishes gradually as the dropsy disappears, although the blood volume is still high. In all stages of the disease, therefore, the dropsy is being actively produced, and it is not a question of the production up to a certain point, and then the gradual absorption of the accumulated fluid after the production has ceased. Absorption is constantly occurring at a certain definite rate, and the accumulation and disappearance of the fluid are entirely due to alterations in the rate of output of lymph. As the anastomoses are established this rate of output falls, and the ascites, therefore, disappears. The ascites is not a stagnant pool of fluid, which is rapidly put out and then slowly absorbed, but at all stages of the disease there is a rapid and continuous circulation of lymph from the blood into the peritoneum and thence back into the blood. The ascitic fluid is thus being constantly changed.

Absorption from Peritoneal Cavity.

We now turn to the question of absorption from the peritoneal cavity, and at the outset it appears self-evident that this process must be occurring at the normal rate at least; otherwise, judging by the amount of fluid which is put out during the day, the animal would soon become tightly distended with ascitic fluid. If the mechanisms whereby absorption is carried out are normal, it is clear that a check would be placed to such a rapid accumulation of fluid, because in the normal animal it is well known that blood serum and other protein solutions are readily absorbed.

In order to test the rate of absorption of a *normal cat* for the ascitic fluid of another cat I did three experiments, and found that the normal cat absorbs at the rate of at least 2 c.cm. per hour, so that in twenty-four hours it would take up from 40 to 50 c.cm. A further point to note is that the amount of solid matter in the residual fluid was precisely the same as that in the ascitic fluid introduced. Absorption occurs at a uniform rate, and the fluid is absorbed as a whole.

The points which have now to be determined are whether the absorptive mechanisms of the *ascitic cat* are in a normal condition, and whether they are as capable of fulfilling their functions as are those of a normal animal.

The normal animal absorbs fluid from its peritoneal cavity in two ways:

1. By its subperitoneal lymphatic plexus and thoracic duct, and
2. By its subperitoneal blood capillaries.

We have, therefore, to inquire into the condition of the lymph flow from the thoracic duct of the ascitic animal, and whether the blood vessels of its peritoneum are capable of absorbing fluid at the same rate as those of the normal animal.

I. *Lymph Flow from the Thoracic Duct.*

The effect upon the lymph flow of *complete obstruction* of the inferior vena cava above the diaphragm is well known. Experimenting on the dog, Heidenhain showed that the lymph flow is immediately increased. This increase may be moderate but sometimes it is enormous—ten to twenty fold. The total solids in the lymph are increased by 1 or 2 per cent. Ligature of the portal vein gives rise to a more moderate increase in the lymph flow, the increase being about five fold. The total solids in the lymph in this case are diminished by about 1 per cent. Starling has proved that the lymph after obstruction of the inferior vena cava comes entirely from the liver, that its increased quantity is due to the very high pressure of blood in the liver capillaries, and that its increased concentration is due to the great permeability of the walls of the liver capillaries as compared with those in other parts of the body. I have repeated these experiments on the cat and have obtained similar results. I have not, however, found such great increases of lymph flow as occur in the case of the dog. The increases which I have noted after ligature of the inferior vena cava have been much smaller, not more than two or three times the normal, and commonly less than this. In all, however small the increase in quantity, the total solids of the lymph were increased by about 1 or 2 per cent.

When the vein is *partially obstructed* above the diaphragm, there is no immediate effect upon the lymph flow. The animals with ascites may be divided into two groups with respect to their lymph flow: The first group includes those during the first few days after the vein is constricted in which the venous pressures are normal or moderately raised, and in which the blood volume is normal or not materially increased; in this group the lymph flow is within normal limits. The second group includes all those at a later date in which the venous pressures are high, and in which the blood volume is increased; in this group the lymph flow is always increased. In both groups the rate of lymph flow does not depend upon the amount of ascites present.

Cause of Increased Lymph Flow.

We see, therefore, that when the disease is fully established there is a considerable increase in the lymph flow from the thoracic duct, and we have now to inquire whether this excessive flow is indicative of increased rate of absorption from the peritoneal cavity, or whether it is due to increased rate of lymph production.

If the increased flow of lymph were indicative of *increased rate of absorption* from the peritoneal cavity, the rate of flow should correspond with the amount of ascitic fluid present, because there is no other reason than the accumulation of peritoneal fluid which would give rise to the increased lymph flow. But I have shown that, although the thoracic duct is capable of carrying away fluid much more rapidly than normal, the lymph flow is not increased during the first few days when large amounts of ascitic fluid accumulate. Also, in the later stages the flow may be greatly exaggerated, although there are only small amounts of ascitic fluid present. It is quite true that the lymph flow increases at the period when there is an increase in the ascites, but that the two conditions depend upon some common factor and not the one upon the other is apparent from the comparative study of individual cases.

That injections of fluid into the peritoneum do not give rise to an increase in the lymph flow was shown first by Orlow, and later by other observers. Saline solutions are absorbed readily, but during the process there is no increase in the lymph flow. In the human being, however, the pressure of ascitic fluid not uncommonly reaches a considerable height. An abdominal pressure of 100 mm. Na_2SO_4 solution was recorded by Starling and myself in a case of ascites in a dog with heart failure. The abdomen contained 2,310 c.cm. of ascitic fluid and the lymph flow was greatly increased. During the first ten minutes 30 c.cm. of lymph (about ten times the normal) were obtained and when the flow settled down to a steady rate it remained about five or six times above the normal. After tapping the abdomen and emptying the cavity the lymph flow still remained at its high level, showing that the rapid flow was not due to draining away of the peritoneal fluid, but still it might have been due to drainage of distended lymph spaces. In order to determine whether fluid at high pressure was rapidly carried off by the thoracic duct from the peritoneal cavity I performed two experiments, which gave identical results, and showed that there was no increase in the lymph flow, although fairly large amounts of fluid were absorbed.

A distinction must be drawn between absorption by the lymphatics from the peritoneal cavity and from the tissue spaces. The peritoneal cavity is enclosed by a definite membrane, and is in intimate relation with a subperitoneal plexus of lymphatics, but there is probably no direct communication between them. It is regarded at the present time that the endothelial cells of the peritoneal membrane are everywhere in close contact with each other, and that there are no permanent openings between them. Fluids and solid particles, however, pass with ease through the interstices between the cells. The force causing the movement of such fluids and particles into the subperitoneal lymphatics from the abdominal cavity is derived from the movements of respiration, and more particularly of the diaphragm, owing to the arrangement of its lymphatics. Solid particles are no doubt chiefly carried through by leucocytes. The composition of the fluid on each side of the peritoneal membrane is practically the same, so that there is no force tending to osmosis or diffusion. Absorption from the peritoneum is thus a relatively slow process, and it is possible that if the respiratory movements are interfered with by an excessive accumulation of fluid the process of absorption by the lymphatics will be correspondingly slow. The mere accumulation of fluid in the abdominal cavity would not, therefore, increase the outflow of fluid from it. A rapid output of lymph from the capillaries would, on the other hand, distend the lymph spaces, and materially raise the pressure in them, thus increasing the outflow of lymph. This is particularly true of the lymph spaces of an organ such as the liver, which is enclosed in a capsule, so that any increase of lymph production in it more easily raises the pressure in its lymph spaces, and increases the flow of lymph from its lymphatics. So long as the tissue fluid remains inside the abdominal organs it is absorbed comparatively quickly by the lymphatics, but when it drains out into the peritoneum it is lost to the lymphatics, and a certain time must elapse before it can be absorbed in the usual manner. The increased lymph flow of the ascitic animal is therefore not indicative of increased rate of absorption from the peritoneal cavity, but such absorption by the lymphatics takes place at the same rate at all stages of the disease, and also at the same rate as in the normal animal. There is no increase in the rapidity with which fluid is drained off during the period of subsidence of the ascites from the peritoneum or from the lymph spaces, which might be supposed to have been overfilled.

If the increased lymph flow of the ascitic animal is not dependent upon increased rate of absorption of the ascitic fluid, it must be an index of *increased lymph production.*

We have already seen that, at the period at which the lymph flow becomes increased, the ascites attains its maximum height both in quantity and rate of production.

The latter depends upon the rate of production of lymph, so that the increased lymph flow coincides with increased lymph production. At this time, also, a general plethora is established, the pressures in the portal vein and inferior vena cava rise and the liver swells to an increased extent. It was first shown by W. Müller, and is now universally recognized, that in artificial plethora there is increased lymph production and increased lymph flow from the thoracic duct. This increased lymph production takes place chiefly in the abdominal organs, which are thus responsible for the increase in the flow of lymph. There is therefore no doubt that the increased lymph flow and the increase in the ascites are directly dependent upon the plethora, and that the lymph comes from the abdominal organs, particularly the liver.

The question which now requires an answer is why there is no increase in the lymph flow from the liver in incomplete obstruction of the inferior vena cava until the condition of plethora is established, whilst there is a considerable output of fluid into the peritoneum, giving rise to ascites. The explanation appears to lie in the different degrees of tension in the distended liver. The flow of lymph is constant from the lymphatic radicles to the thoracic duct and thence into the veins. There must, therefore, be a gradual rise of pressure as we follow the lymph stream up into the liver. The higher the pressure in the lymphatics of the liver the more rapid must be the flow of lymph. In the early stages of narrowing of the inferior vena cava the liver distends owing to the rise of venous pressure behind the obstruction, but we have seen that this rise, although enough to dilate the vessels of the liver and fill out that organ with blood, is really only trifling. The vessel walls are altered and lose their tone, so as to remain dilated under a pressure which is normal or only slightly raised. The lymph production is increased owing to nutritive changes in the capillary walls, but the pressure in the lymphatics does not rise high enough to materially increase the lymph flow from the thoracic duct. The nutrition of all the tissues of the liver suffer, and lymph drains out through the capsule into the peritoneum. When plethora is established the venous pressures rise to a high level, the liver enclosed in its capsule distends further, and the tension within it becomes considerable. There is a further increase in the lymph production, and the lymph finds an easier exit through the lymphatics than through the capsule. The condition now has become somewhat similar in many features to that immediately resulting from complete obstruction of the inferior vena cava, in which there occurs a rapid rise of pressure in the liver, a great increase in the lymph flow, and slight ascites. Ascites will accumulate without any material rise of pressure in the liver, but the rate of the lymph flow is directly dependent on the pressure in that organ. In the case of complete obstruction the percentage of total solids in the lymph is increased, this being due to the fact that the lymph is entirely derived from the liver. In the ascitic animal there is no increase in the solids of the lymph although the flow is increased. This is what might be expected if the increased lymph flow resulted from plethora, because in this condition the lymph comes not only from the liver but also from the intestine, the lymph of which is of less protein concentration than normal lymph. The lymph is of no greater concentration than the normal because it is a mixture of intestinal and liver lymph. Before plethora is established, and when the lymph flow is normal, the percentage of total solids is also within the normal limits.

II. *Absorption by the Subperitoneal Blood Capillaries.*

For reasons which I have already stated, I assumed that increased absorption was taking place by the capillaries at the periphery of the body, and we have now to inquire whether this assumption is justified by experiment. The blood from the capillaries of the parietal peritoneum passes into the veins of the abdominal wall, so that these capillaries form part of the system of vessels which are situated at the periphery. If, therefore, it can be proved that an increased absorption is taking place from the peritoneum directly into the blood, we shall be justified in concluding that our assumption is correct, because it is obvious that the visceral peritoneum, which is putting out lymph at such a rapid rate, cannot be held responsible for increased absorption.

For many years it had been known to physiologists that the blood vessels were able to absorb fluids and dissolved substances, but due prominence was not given to this method of absorption until it was definitely proved by Starling and Tubby that absorption of colouring matters in solution from the serous cavities took place rapidly by the blood vessels. They found that the residue of the solution, which they had introduced into the serous cavity, on withdrawal contained protein, and concluded that the process at work was really one of interchange between the fluid in the cavity and the blood, each taking from the other that constituent which it did not possess.

Later researches by Leathes and Starling shewed that this absorption was due to osmosis, and that there was no question of any active absorption by the endothelium because there was no material difference in the process when the endothelium was injured by sodium fluoride or by scalding. They proved that not only was water absorbed by osmosis, but also sodium chloride by diffusion.

Osmosis and diffusion will thus account for the absorption of saline solutions by the blood vessels up to a certain point—namely, that at which there is equalization of the amounts of the various salts on each side of the membrane when osmotic equilibrium is established. The osmotic pressure of the blood proteins, as Starling has pointed out, must also be taken into account, the force of attraction of which for extravascular fluid varies inversely as the concentration in protein of the latter. One would therefore expect absorption of fluid by the blood vessels to still continue until increasing protein concentration of the peritoneal saline solution reduced the absorbing force due to the difference of osmotic pressures on each side of the membrane to the level of the hydrostatic pressure in the capillaries. Further than this osmosis and diffusion will not account for the absorption of ascitic fluid by the capillaries.

Normal Cat.

Our first task is, therefore, to find out whether the ascitic animal is capable of absorbing salt solution by its subperitoneal blood capillaries, and, if so, what is the rate of this absorption as compared with the normal. In order to do this I have employed Starling's method of injecting a coloured solution into the peritoneum, and first, in order to obtain a standard of the normal rate of absorption in the cat, I repeated the experiments on the normal animal. As the urine is not uncommonly suppressed in these experiments, instead of noting the colour of this fluid as an index of the colour of the blood, small amounts of blood were withdrawn from the femoral artery at short intervals.

Three experiments were done, with the same results in each case. The blood serum was distinctly tinged blue in about six minutes after introducing the fluid into the peritoneum, and in a few minutes later it was fairly dark blue. The lymph was tinged in from fifteen to twenty minutes, and was fairly dark blue in thirty to forty minutes. The urine was also coloured blue when it was excreted. In no case was the lymph flow increased, and it was never darker than the corresponding sample of blood.

These experiments are in entire agreement with those of Starling, and show that coloured saline solution is absorbed rapidly from the peritoneum of the cat by the blood vessels, being distinctly visible in the blood in about six minutes, and in the lymph in about fifteen to twenty minutes.

Ascitic Cat.

We now turn to a consideration of absorption by the blood vessels of the ascitic animal. I have done two experiments: the first animal had had its inferior vena cava narrowed twenty-four hours, and the second one twenty-seven days, previously. The experiments were conducted exactly as described above. In the case of the first cat the lymph flow, as is usual with animals on the day after operation, was normal. In four minutes after the indigo solution had been introduced the blood showed a distinct tint of blue which rapidly darkened, but the lymph was not stained until about sixteen minutes had elapsed after the first tint appeared in the blood. The second cat, which had been operated upon twenty-seven days previously, was well nourished and healthy. The lymph flow of this cat was increased, as is usual with animals at the end of a month when the blood volume is increased. The blood was tinged blue in four minutes, and the lymph in ten minutes, after the coloured fluid was introduced. The colours of the blood and lymph increased in intensity, the blood at first being the darker of the two.

It is quite evident from these two experiments that the ascitic animal is quite as capable of absorbing coloured saline solution by its subperitoneal capillaries as the normal animal, and that the absorption occurs more rapidly, because the blood was tinged in a shorter time in the ascitic than in the normal animal. The rate of osmosis is therefore greater than in the normal animal, and from this we are justified in drawing the conclusion that the capillaries of the abdominal wall are absorbing fluid from the tissue spaces more rapidly than normal. The same conclusion must be formed in regard to the hind limbs, so that in the whole periphery of the body increased absorption is taking place.

One further fact is noticeable. The lymph of the second animal was coloured in about half the normal time. This is due to the more rapid lymph flow of the animal. It is an additional fact in support of my statement earlier on that the increased lymph flow of the ascitic cat is an index of increased lymph production.

It will be remembered that a portion of the parietal peritoneum is drained by veins opening into the superior vena cava. It might, therefore, be objected to these experiments that the osmotic absorption of fluid really occurred into these vessels and not into those opening into the inferior vena cava, so that the experiments did not prove absorption by branches of that vessel. This question can easily be set at rest by ligaturing the superior vena cava before the coloured fluid is run into the peritoneal cavity, so that there can be no doubt that the fluid is absorbed by branches of the inferior vena cava. I therefore performed two experiments of this nature. In the first cat the inferior vena cava had been constricted three days. A cannula was connected with the thoracic duct, and the superior vena cava ligatured between the entrance of the azygos vein and the auricle. The lymph flow was normal. In two minutes the blood serum was tinged blue and in four minutes from the beginning its colour was markedly blue. The lymph was not tinged till twenty-one minutes after the fluid was introduced. The second cat was also a three days cat. The same procedure was adopted. The lymph flow of this animal was a little above the average. In two minutes the blood serum was tinged blue as in the first cat. The lymph was tinged in ten minutes.

These two experiments prove that the osmotic absorption of fluid occurs into the blood vessels of the parietal peritoneum when all anastomoses are cut off. It will be noticed that absorption occurs in a third of the normal time when the superior vena cava is ligatured. I have already shown that when this vein is ligatured there is a great rise of pressure in all its tributaries from the accumulation of blood in them, and that simultaneously the pressure in all the tributaries of the inferior vena cava falls owing to abstraction of blood. This is the reason of the increased rate of absorption of the fluid. Ligature of the superior cava relieves the abdominal vessels of some of their blood, with the result that they take in fluid more rapidly. It is thus clear that, when both superior and inferior venae cavae are obstructed together, increased absorption takes place in the extremities and in the body wall, whilst increased output is occurring in the central parts of the body. This must also take place in uncompensated heart disease, which I have shown to be precisely similar in its results to the simultaneous obstruction of the superior and inferior venae cavae.

So far we have considered the rate of absorption by osmosis at an early stage of the process, and we have now to study the rate at which it occurs *during the later stages* until absorption is complete. This was investigated by removing all the ascitic fluid and replacing it by normal saline solution, which was withdrawn at different periods of time, and examined in regard to its amount and its concentration in dissolved solids as compared with the normal animal. .

On comparing the rates of absorption of saline solution in the normal and the ascitic cat I have found that during the first hour almost the same amounts are absorbed, the ascitic cat absorbing rather more than the normal, but the residual fluid in the ascitic cat contained a percentage gain of solid matter which was six times as great as that of the normal cat. In the next experiment the absorption was allowed to go on for eight hours: 70 c.cm. of saline solution were introduced into a normal cat and at the end of eight hours only 3 c.cm. could be recovered from the peritoneum; the percentage of total solids in it amounted to 4.7; 45 c.cm. of ascitic fluid were removed from the peritoneal cavity of a dropsical cat and 70 c.cm. of saline solution introduced; at the end of eight hours 45 c.cm. were recovered from the peritoneum. The rate of absorption in this cat had fallen off two and a half times as quickly as in the normal animal. The percentage of total solids in the residual fluid was 6.6. The original ascitic fluid of the cat contained 7.9 per cent. of total solids, so that in eight hours the animal had almost made up its original percentage of solid matter in the peritoneal fluid, and the volume of the fluid was precisely the same as the original amount of ascitic fluid. In another ascitic cat of two weeks' standing 30 c.cm. of fluid were removed containing 6.5 per cent. total solids. The amount of saline solution introduced was 49 c.cm., and after twenty-four hours 22 c.cm. of fluid were removed containing 6.5 per cent. solids.

One may state generally that certainly within twelve hours the introduced fluid will have become about the same as that originally withdrawn both in amount and composition. We see, therefore, that in the ascitic cat the interchange of solids between the blood and the saline solution contained in the peritoneum is a much more rapid process than in the normal animal. The process of osmosis is more rapid in the ascitic than in the normal animal during the early stages, but it is interrupted much more quickly in the ascitic animal by the more rapid output of solid matter, so that in the later stages absorption of fluid becomes much slower, and osmotic equilibrium is established long before the fluid can possibly be absorbed.

We have now to inquire into the *origin of the protein* which accumulates in the peritoneal fluid. The presence of salts which collect in the fluid are quite easily accounted for by diffusion, so that the partial pressure of each saline constituent of the blood becomes equal on each side of the separating membrane. The presence of the protein cannot be thus easily explained, as protein is indiffusible. In the first place, I must point out that we are dealing with two sets of blood vessels—those of the viscera, in which output has overbalanced absorption, and from which the ascitic fluid originates. and those of the parietal peritoneum which are absorbing more quickly than normal—so that we must distinguish two separate processes: osmotic absorption of fluid and equalization of partial pressure of salts by diffusion in the case of the capillaries of the parietal peritoneum, and increased output of lymph from those of the viscera. It is the increased output of lymph into the saline solution which rapidly brings its concentration in protein and salts up to that of the original ascitic fluid, and so arrests osmosis and diffusion in the case of the parietal peritoneal vessels. This arrest of osmosis occurs at a certain stage in each animal, depending entirely upon the rate of output of lymph.

The protein thus originates in the lymph which is being rapidly produced by the viscera, and for this reason the fluid becomes of the same composition as the original ascites.

When salt solution is introduced into the peritoneum of the normal animal protein appears in it for the same reason—namely, that there is normally a very slow output of lymph into the peritoneum, which is being as rapidly absorbed, so that this structure is kept slightly moist, and there is no accumulation of fluid in it. It is this normal lymph production which accounts for the appearance of protein in the peritoneal saline solution. In support of this hypothesis may be mentioned the fact, noted by Gray and myself, that sometimes in the normal hutch-fed rabbit, and commonly when it is pregnant and the muscles are flabby and the abdomen is full, ascitic fluid is present. We showed that there were no pressure changes in the circulation of these ascitic animals. The ascitic fluid of these rabbits appears to be normal tissue fluid, the rate of production of which is probably normal, but absorption by the lymphatics is impeded owing to the respiratory movements and action of the abdominal muscles being less effective in this direction than are those of a wild animal, in which the abdomen is taut and the movements active and vigorous.

Finally, we arrive at the question whether absorption entirely ceases by the blood vessels of the peritoneum when osmotic equilibrium is established between the ascitic fluid and the blood. This entirely depends upon whether protein is absorbed by the capillary wall or not.

An increased absorption of fluid may be occurring by the capillaries of the abdominal wall, although they do not take up the ascitic fluid from the peritoneum. The explanation of this is that the protein concentration of tissue fluid is kept low by the tissue cells using up the protein during their metabolic activities, whilst the ascitic fluid remains of the same protein concentration. Until proof is forthcoming that absorption of protein may occur by the capillary wall, we must assume that when the ascitic fluid attains osmotic equilibrium with the blood plasma the ascites is entirely absorbed by the lymphatics.

Summary.

1. The accumulation of fluid in the peritoneal cavity following an incomplete obstruction of the inferior vena cava in the chest, or in uncompensated heart disease, is due to an increased output of lymph from the viscera, more especially the liver. This increased output is constantly occurring at all stages of the disease; an increase in the amount of fluid is due to an increase in the rate of output, and the disappearance of the fluid is due to a gradual falling off of the rate of output.
2. Absorption from the peritoneal cavity by the lymphatics is occurring at all stages at the normal rate, a check being thus placed to an excessive accumulation of the ascitic fluid. The fluid is therefore being continuously renewed, and there is a constant circulation of lymph from the blood into the peritoneal cavity and back again to the blood by the thoracic duct.
3. Increased absorption of tissue fluid is taking place in the abdominal wall and hind legs, as in the fore part of the body, but it has not been proved that the ascitic fluid is absorbed as such by the capillaries of the parietal peritoneum. There is no evidence to show that the endothelial cells of the peritoneum actively absorb the fluid.
4. The increased lymph flow from the thoracic duct observed at a certain stage in the course of the dropsy is an index of increased lymph production in the abdominal organs owing to the plethora.

Bibliography.

Bolton and Gray: British Medical Journal, March 18th, 1911.

Bolton and Starling: *Heart*, 1910, vol. i, No. 4, p. 292.

Heidenhain: *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. xlix, S. 209.

Leathes and Starling: *Journ. Physiol.*, 1895, vol. xviii, p. 106.

Müller, W.: *Lect. Gen. Path.* (Cohnheim), New Syd. Soc. Trans., 1889, vol. i, p. 419.

Orlow: *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lix, S. 170.

Starling: *Journ. Physiol.*, 1895-96, vol. xix, p. 312; and 1894, vol. xvi, p. 224.

Starling and Tubby: *Journ. Physiol.*, 1894, vol. xvi, p. 140.

No. 3

pp. 290–326.

Journal of Pathology and Bacteriology, Vol. XX.

FURTHER OBSERVATIONS ON THE PATHOLOGY OF CARDIAC DROPSY.[1]

By Charles Bolton, M.D., D.Sc., F.R.C.P., *Physician to University College Hospital, London, W.C.*

From the Graham Research Laboratories, University of London.

Introduction.

The present research is a continuation of an investigation into the pathological processes occurring in uncompensated heart disease, which was commenced some years ago, the first communication on the subject being published in this Journal in 1903 [1].

The original idea was to produce experimentally an uncompensated condition of the circulation in order to study the exact alterations which occur in the circulation of the blood, in its volume and composition, in the composition and flow of the lymph, and other problems connected with this morbid state, it being quite impossible to obtain precise information on these points by observations on the human subject. Until such information was definitely acquired it was considered that our knowledge of the pathology of cardiac dropsy would advance no further towards a satisfactory solution.

RECAPITULATION OF THE AUTHOR'S PREVIOUS RESULTS.

The method adopted in my earlier experiments consisted in *constricting the pericardium* by means of sutures so that the diastolic expansion of the heart was impeded. An uncompensated condition of the circulation resulted, the volume of blood in the veins was augmented and in the arteries diminished, and dropsy supervened (1903 [1]). I then found that precisely the same circulatory conditions resulted when I *narrowed the superior and inferior venæ cavæ together* at their entrance into the heart (1907–1909 [2]). My experiments both in the case of pericardial constriction and obstruction of the large venous trunks were concerned with the circulatory alterations occurring during the first twenty-four hours following the operation, and the main results which I observed during this period may be briefly expressed as follows.

---

[1] [Received January 10, 1916.] The expenses of this research have been defrayed by grants from the Royal Society and the Graham Research Fund.

Results of Constriction of Pericardium or Narrowing of both Superior and Inferior Venæ Cavæ.

1. The immediate result is a general rise of pressure throughout the whole venous system extending to the small venous radicles, and a marked fall of the mean arterial blood pressure.

2. A gradual swelling of the liver and an increasing engorgement of the splanchnic veins and of the large veins of the trunk, together with their immediate tributaries, rapidly appear. Ascites, hydrothorax, and œdema of the mediastinum and retroperitoneal tissue make their appearance, and in two or three hours the fluid is measurable in amount.

3. The venous pressure, however, does not remain for long at the level it had immediately assumed. It commences to fall, and eventually reaches the normal level or thereabouts in the space of an hour or an hour and a half. The arterial pressure remains at the low level to which it had fallen or even a little lower.

4. When the animal has recovered from the anæsthetic and resumed its normal conditions of life the pressures undergo further changes. The venous pressures in the portal system and in the large veins of the trunk and their immediate tributaries gradually rise until they attain the heights at which they stood immediately after the operation. This rise of pressure may be completed within about four hours, but in many of the cases twenty-four hours later the pressures are still within normal limits. The venous pressures in the peripheral parts of the circulatory system (periphery of body and extremities), however, after they have once fallen, do not rise again; in those animals in which the venous pressure in the central portions of the trunk are again raised, the pressures in the extremities and periphery of the trunk are normal; and in those in which the pressure in the trunk are still normal, the pressures in the extremities are subnormal. The arterial pressure follows the venous pressure in the large veins of the trunk. Those animals in which these venous pressures are raised have normal arterial pressures, and those in which they are at the normal level have subnormal arterial pressures.

5. The presence and the amount of the dropsy are independent of the height of the venous pressures; the dropsy commences whilst these pressures are normal, and is present twenty-four hours after the operation whether they are normal or raised.

6. My final interpretation of the circulatory changes occurring up to the end of the first twenty-four hours was, that the distensibility of the liver, portal area, and large veins of the trunk is responsible for the fall in the venous pressures; and that the subsequent rise of pressure in the veins and in the arterial system is due to vasomotor overaction, the pressure in the peripheral portions of the body and limbs remaining normal or subnormal.

There is, therefore, a depletion of the peripheral parts of the circulatory system and an accumulation of blood in the central portions of the body, the actual height of the pressures depending on the degree of action of the vasomotor centre, so that a *relative* alteration in the venous pressure levels results, the pressure in the central portion of the trunk being raised *relatively* to that in the peripheral portion and in the extremities.

The amount of blood in the body is definitely limited, and therefore if one portion of the vascular system is increased in capacity the pressure can only be kept at the normal or a raised level by vasomotor overaction.

The vascular constriction occurs automatically by stimulation of the vasomotor centre by the deficient blood supply to the brain. The resulting rise in arterial pressure ensures a fuller supply of blood to the brain, and the rise in venous pressure a fuller supply of blood to the heart.

There is an increased output of lymph with diminished absorption by the capillary vessels in the congested areas, giving rise to dropsy. An increased absorption of lymph occurs in the depleted areas in order to restore the normal volume of blood in these parts.

Cardiac dropsy is thus seen to be really a form of local dropsy, the locality being determined by the distensibility of a certain portion of the vascular system. Dropsy occurs in this locality only; in other parts of the body increased absorption of lymph takes place. The assumption of the erect posture in man relieves the congestion of the portal system and central portions of the trunk at the expense of the lower part of the body and the legs, so that the dropsy of the peritoneum and central portions of the body is lessened or at a minimum, and œdema of the legs is produced.

With regard to obstruction of the *two main venous trunks separately*, I found that narrowing or even complete ligature of the superior vena cava alone produced no change whatever in the circulatory system of the body as a whole, a local œdema affecting the mediastinum and pleural cavities simply resulting. Narrowing of the inferior vena cava alone, however, produced general circulatory changes similar to those resulting from pericardial constriction or narrowing of both main venous trunks, the only difference being that there was no preliminary venous congestion of the head and fore part of the body, and no hydrothorax or œdema of the mediastinum resulted.

Results of Narrowing of the Inferior Vena Cava alone.

I found that precisely the same adaptive mechanisms as are described above were brought into play as the result of partial obstruction of the inferior vena cava above the diaphragm.

No change whatever was to be noted until the vein was narrowed down to three-fifths of its normal diameter. This is owing to the fact that the veins and capillaries are not filled to distension and will hold a certain amount of blood before the pressure within them is raised. Complete ligature of the vein causes a sudden fall of arterial pressure to a few millimetres above the zero line, and the animal dies in a few hours.

On narrowing the vein to any size below three-fifths of its diameter, the venous pressure behind the obstruction goes up, the rise extending to the small venous radicles of the hind-limbs and intestines, and the general arterial pressure falls. The venous pressure subsequently falls to normal or thereabouts owing to dilatation of the large veins of the trunk and of the liver and portal system. The venous pressure of the trunk and the arterial pressure subsequently rise, but the pressure in the veins of the hind-limbs remains low. Ascites and œdema of the retroperitoneal tissue result. The only difference between the actual circulatory conditions established and those described above is, that the territory drained by the superior vena cava is not included in the congested area, so that blood is abstracted from the whole forepart of the body. The absorptive area, therefore, includes the whole forepart of the body in addition to the peripheral parts of the abdomen and extremities. The mechanisms involved are, therefore, precisely the same, the difference being one of degree only.

RESULTS OF FURTHER EXPERIMENTS.

The present series of experiments examines the changes over several weeks, and the method which I have employed is that of narrowing of the inferior vena cava alone. My reasons for adopting this method were, that the operation is much more easily and quickly performed than that of constriction of the pericardium, the degree of obstruction can be accurately measured, and only now and again have I found that the animal dies from the effects of the operation. Moreover, the method possesses the distinct advantage that by the establishment of anastomoses the animal finally recovers from its dropsy. One can, therefore, study the various processes occurring, not only during the onset and gradual increase of the dropsy, but also those occurring during its gradual subsidence.

The subject will be considered under the following headings :—

1. Method.
2. Blood volume, hæmoglobin percentage, and vascular pressures of the normal cat.
3. Course of the ascites.
4. Output of lymph in the congested area.
5. Blood volume of the dropsical animal.
6. Hæmoglobin content of the blood in the dropsical animal.
7. Vascular pressures of the dropsical animal.
8. The intake of fluid and the excretion of urine in the dropsical animal.
9. The occurrence of jaundice.
10. Summary and conclusions.

1. *Method.*

The operation for partial obstruction of the inferior vena cava has been already described in a former paper (1909 [2]), to which the reader is referred for the details. The animals used were cats, and in every operation or experiment the animal was anæsthetised with ether. The vein was in all cases obstructed in the chest just below the heart, artificial respiration being maintained during the operation through an intubation tube. The vein was narrowed down to a diameter of 3 mm. I have found the diameter of the vein distended at the normal pressure to vary a little in different cats, so that the degree of narrowing was not exactly three-fifths of the original diameter in all cases. For the purposes of these experiments it was unnecessary to endeavour to produce the same relative degree of obstruction in each case, and, in fact, such an attempt could not succeed, as one cannot foretell what the exact size of the vein will be in any particular cat.

The percentages of hæmoglobin and blood corpuscles were estimated with the Haldane-Gowers hæmoglobinometer and the Thoma-Zeiss hæmocytometer. It was found that alterations in the amount of hæmoglobin corresponded with similar alterations in the same direction in the number of corpuscles, so that in most of the experiments the hæmoglobin alone was estimated in order to save time and labour. Blood from the head was obtained by pricking the marginal vein of the ear; from the hind-leg by pricking the pad of the paw; and from the abdominal vessels by making a small incision through the abdominal wall, carefully drying the surface of the liver and pricking it with a needle.

The volume of the blood was obtained by the washing-out method, the animal being anæsthetised with ether. A sample of blood was first removed and diluted a hundredfold with water. The animal was then bled from the carotid artery, and at the same time warm Ringer's fluid was run into a vein so as to keep up the blood pressure and maintain the action of the heart as long as possible. The blood was collected in citrate solution. By this means the heart would often go on beating until the fluid issuing from the carotid was almost colourless. The next procedure was to insert a cannula into the descending aorta and wash out the abdomen. A certain amount of blood-stained fluid was always obtained from the inferior vena cava by this means, however thorough had been the original washing-out of the vessels. Similarly, a small amount could be obtained from the head and upper limbs by washing-out through the ascending aorta. The washings were all mixed together and the resulting fluid diluted until it was exactly the same colour as the hundredfold dilution of the sample of the blood. The total volume of the blood was then calculated.

The blood pressures were taken in the carotid artery, the external jugular vein through the posterior auricular vein, the portal vein, the inferior vena cava (lower end), and in a vein of the hind-leg, as described in the papers referred to above.

The rate of output of lymph was determined at different stages in the course of the ascites by removing all the abdominal fluid with a pipette and absorbent cotton wool, and noting the amount which had accumulated at the end of the next twenty-four hours.

The animals recovered well and lived healthy lives. They were fed on cat's meat and milk, and the total amount of fluid consumed and of urine passed was carefully noted each day.

2. *Blood Volume, Hæmoglobin Percentage, and Vascular Pressures of the Normal Cat.*

(*a*) *Blood volume.* By the method described above I have found that the blood volume in cubic centimetres of the normal cat is about one twenty-fifth of its body weight in grms.

The results of ten determinations are given in Table I.

Taking the blood volume as one twenty-fifth of the body weight in grms. expressed in c.c., the greatest error in the above experiments was about 8 c.c.

(*b*) *The percentage of hæmoglobin* is not the same in all cats; for example, cat 4 had 100 per cent. Hb, cat 6 had 64 per cent. Hb, and cat 8 had 90 per cent. Hb. The percentage of hæmoglobin, therefore, bears no simple relation to the blood volume.

In all experiments dealing with alterations in the percentage of hæmoglobin it is thus necessary to determine in the first place what is the normal percentage of the particular cat under investigation. The blood obtained from an ear vein contains the same amount of hæmoglobin as that obtained by pricking the pad of the foot, but that from the liver appears to contain if anything a trifle more; for example, one determination showed: ear, 90 per cent.; foot, 90 per cent.; liver, 92 per cent. The number of red blood corpuscles also varies in different cats, usually being from 10 to 12,000,000 per c.mm.

TABLE I.

No.	Weight.	Blood Volume determined by Experiment.	Blood Volume calculated as one twenty-fifth of the Body Weight in grms. (expressed in c.c.).
1 . .	2085 grms.	85 c.c.	83·4 c.c.
2 . .	2460 ,,	91·1 ,,	98·4 ,,
3 . .	2500 ,,	99 ,,	100 ,,
4 . .	2665 ,,	114 ,,	106·6 ,,
5 . .	2790 ,,	111 ,,	111·6 ,,
6 . .	2800 ,,	117·6 ,,	112 ,,
7 . .	2835 ,,	117·5 ,,	113·4 ,,
8 . .	3200 ,,	120 ,,	128 ,,
9 . .	3520 ,,	140·2 ,,	140·8 ,,
10 . .	3775 ,,	147·8 ,,	151 ,,

(*c*) *The venous pressure* at the lower end of the inferior vena cava is about 97 mm. $MgSO_4$ solution (sp. gr. 1046); in the foot about 160 mm. $MgSO_4$ solution; in the portal vein about 104 mm. $MgSO_4$ solution (usually a few millimetres above that at the lower end of the inferior vena cava); and in the external jugular vein (posterior auricular) about 79 mm. $MgSO_4$ solution. The arterial pressure is usually high, and not uncommonly reaches 180 to 200 mm. Hg.

3. *Course of the Ascites.*

As stated above, *the ascites appears during the first few hours* after the obstruction of the inferior vena cava is produced. It gradually increases in quantity up to a certain amount, and then slowly disappears. The cause of the disappearance of the ascites is the establishment of anastomoses. The anastomotic veins of the abdominal wall begin to distend at once, and they gradually increase in size till fully developed. The portal anastomoses are more slow in their development. For an account of these anastomoses the reader is referred to a recent paper (1914 [3]), in which the case of a monkey is described in which the obstruction of the inferior vena cava went on to complete obliteration. The ascites, which was proved to be present by abdominal section, also disappeared in this case, so that the general statement may be made that to whatever degree the inferior vena cava is narrowed the resulting ascites will disappear with the completion of the establishment of anastomoses.

The length of time the dropsy persists depends upon the exact degree of constriction, and perhaps also upon certain minor and as yet unascertained factors. In most of the cases in which this point was examined the dropsy disappeared from the seventh to the twelfth week. In five cases a trace only was found in the sixth, seventh, seventh, eighth, and eleventh weeks. In four cases—in the eighth, eighth, tenth, and seventeenth weeks—12 c.c., 3 c.c., 2 c.c., and 15 c.c. were found respectively. The last case is the only one in which the duration of the dropsy extended over a longer period than three months. In four animals killed at the end of the eleventh, thirteenth, sixteenth, and twenty-first weeks no ascites was found. All the animals examined at shorter periods than six weeks showed varying amounts of fluid in the abdomen.

It is difficult to estimate the time the dropsy takes to *attain its maximum height and the time of subsidence*, because the amounts of fluid vary in different animals at the same stage; in the lesser degrees of constriction smaller amounts of fluid are found and it subsides quicker. In fourteen animals the amounts of fluid present at the end of the first twenty-four hours varied from 8 to 35 c.c., in one it was 45 c.c., and in another 72 c.c. Thus, although large amounts may be found, moderate amounts are the rule during the first twenty-four hours. The largest amounts are found after the first twenty-four hours to the end of the fifth week.

In six cases the amounts were found as detailed in Table II.:—

TABLE II.

Time.	Amount.
3rd day	84 c.c.
8th ,,	100 ,,
14th ,,	80 ,,
27th ,,	100 ,,
32nd ,,	90 ,,
35th ,,	150 ,,

In other cases during this period, however, the amount may be quite small, from a trace up to 15 c.c. After this time no large amounts of fluid are found.

It is impossible to measure the amounts of ascites at different stages in the same cat. An attempt was made to do this by judging the amounts found on making a small abdominal incision.

The results are given in Table III.:—

TABLE III.

Experiment.	Time.	Amount.	Time.	Amount.	Time.	Amount.
16	6 days	Considerable	4 weeks	100 c.c.	...	...
19	14 ,,	,,	5 ,,	150 ,,	...	...
20	...	...	3 ,,	Large	7 weeks	Trace
21	...	...	17 days	,,	8 ,,	12 c.c.
22	...	...	24 ,,	Moderate	11 ,,	Nil
23	6 ,,	Considerable	...	...	7 ,,	Trace
24	13 ,,	Small	...	...	16 ,,	Nil
25	8 ,,	Large	...	...	17 ,,	15 c.c.
28	...	...	4 weeks	Enormous	11 ,,	Nil
32	13 ,,	Small	3 ,,	5 c.c.	...	...
33	13 ,,	Large	4 ,,	40 ,,	...	...
35	...	...	3 ,,	Moderate	6 ,,	Trace
36	14 ,,	Very large	...	...	8 ,,	3 c.c.
39	6 ,,	Considerable	4 ,,	Much more	11 ,,	Small
40	15 ,,	Very large	...	...	10 ,,	2 c.c.

Considering all these results very broadly, they show that the dropsy is at its height from the first to the fifth week after the obstruction is produced, but that after this period it gradually declines. The times no doubt differ very much in individual cats, but probably in the majority the dropsy is rapidly and chiefly produced in the first week, and then slowly increases up to three or five weeks, or remains at a high level to this time, after which it slowly subsides.

4. *Output of Lymph in the Congested Area.*

It was ascertained that at all stages of the disease, whether during the rise or the fall of the dropsy, the output of lymph in the congested area was greater than normal, so that the presence of ascitic fluid was always dependent upon an increased output of lymph. However large or small the amount of fluid present, and whatever the stage of the disease, a reaccumulation of the ascites was found twenty-four hours after the abdomen had been completely emptied. In all stages of the disease, therefore, the dropsy is being actively produced, and it is not a question of the production up to a certain point and then the gradual absorption of the accumulated fluid after the production has ceased. Whenever ascites is present increased formation of lymph is taking place, but at the point when the ascites commences to disappear the rate of formation has begun to fall off, till finally the lymph is produced in normal amount with the disappearance of the ascites.

The amount of reaccumulated ascites twenty-four hours after the abdomen has been emptied is shown in eight cases (Table IV.):—

TABLE IV.

Time of Removal of Fluid.	Amount Removed.	Amount present Twenty-four Hours Later.
2 days	27 c.c.	30 c.c.
3 ,,	84 ,,	20 ,,
8 ,,	100 ,,	40 ,,
2 weeks	80 ,,	95 ,,
4 ,,	100 ,,	20 ,,
5 ,,	150 ,,	45 ,,
7 ,,	Trace	Trace
7 ,,	Trace	Trace

The amount of reaccumulated fluid during the first five weeks is considerable, but when the dropsy is disappearing the rate of accumulation is very slow. The rate of formation of lymph seems from the above figures to increase up to the second week and then to diminish, but the number of cases here recorded is of course too small to justify the conclusion that the dropsy is most actively produced in the second or third week and then diminishes.

The large amounts of lymph which accumulate in the peritoneum in twenty-four hours must be accompanied by a removal almost equally rapid, but not sufficiently rapid to prevent a gradual accumulation of ascites. The rate of lymph flow through the thoracic duct probably depends on the amount of peritoneal fluid. A rapid circulation of lymph thus appears to be constantly in progress from the blood to the peritoneum, and thence to the blood again. The rate of circulation is determined by the rate of output, absorption always falling short of output whilst the ascites is accumulating. When the ascites is disappearing the rate of output gradually falls, and closely following it the rate of absorption also falls as the ascites becomes less, so that output falls short of absorption during this stage. This theoretical deduction, which I am about to test by experiment, is supported by an observation of Starling and myself (1910 [4]), in which we found that the lymph flow of a dog which had developed ascites as the result of heart disease was greatly exaggerated.

5. *Blood Volume of the Dropsical Animal.*

The idea that the volume of the blood is greater than normal in some forms of dropsy has been held for a great number of years, and in the older literature "plethora" played a not unimportant part.

In the writings of Andral (1836 [5]), for example, a form of dropsy due to plethora is described. Oertel (1884 [6]) was the first to definitely teach that in cardiac insufficiency hydræmic plethora was present. He held that diminished work of the heart leads to an increased quantity of blood in the body, and that this blood contained an increased percentage of water. He did not, however, provide proof of this supposition. Cohnheim (1882 [7]) strongly opposed the idea of plethora, and considered it as proved that a lasting pathological increase of the blood quantum could not occur, since W. Müller and others had shown that, when the blood volume of the dog was increased by transfusion of large amounts of blood, the excess was disposed of in a relatively short space of time. He held the same view with regard to hydræmic plethora, because he and Lichtheim (1877 [8]) had shown that, after injection of large amounts of salt solution, the excess of water was rapidly got rid of by the excretory and secretory glands, and that in order to produce œdema excessive amounts had to be injected. The œdema when produced was not distributed as in human dropsy, but affected merely the glands in question and the abdominal organs. Cohnheim (1882 [7]), however, found that œdema of the subcutaneous tissues was produced by hydræmic plethora if increased permeability of the vessel walls in the part was also present, as, for example, is occasioned by venous congestion of the leg, or by a slight inflammatory lesion, or even by long continued simple hydræmia alone. Starling (1896, 1909 [9]) has maintained on theoretical grounds for many years that an increase in the volume of the blood is a necessary consequence of heart failure, and that without it the high venous pressure necessary to maintain the increased diastolic filling of the heart cannot be maintained. He considers that the first effect of heart failure is to cause a rise of venous pressure in the large venous trunks, but a fall of venous and capillary pressures in the portal vein and in the limbs. The subsequent vasomotor constriction is unable to extend the rise of venous pressure to the capillaries of the intestines and limbs, so that increased absorption of lymph occurs owing to the low capillary pressure and deficient filling of the arterial system. Plethora is thus brought about. He also considers that the rate of absorption of water from the alimentary canal would be increased by splanchnic constriction. Water is retained in the body owing to deficient renal excretion. With regard to the actual cause of cardiac dropsy he is of opinion that it is due to increased capillary pressure in the legs owing to the effect of gravity, acting in conjunction with an alteration in the capillary wall brought about by the stagnation of the blood. The experiments now to be described show that at a certain stage in the condition of circulatory uncompensation an actual increase in the volume of the blood does occur and that it is more dilute than normal. This increase in the blood volume, which is often very small, is, however, merely a secondary phenomenon in the course of events and is not directly concerned in the initiation of the dropsy. My results show five main differences from Starling's deductions.

1. That the initial rise of venous pressure extends through the liver into the portal vein and thence into the venous radicles of the intestines, and also into the venous radicles of the feet. That the capillary pressure in these parts also rises above normal; it falls below normal as the veins dilate, and rises again as the venous pressure goes up but not to its original height, owing to vasomotor constriction, particularly in the feet. It rises again later when plethora is established.

2. That a high venous pressure can be maintained by vasomotor constriction and without any increase in the total blood volume.

3. That the increased absorption of lymph, largely responsible for the hydræmic plethora, does not occur uniformly as a result of the fall of arterial pressure, but only in the depleted areas; in the congested areas increased output only of lymph occurs. In other words, fluid is absorbed in one part of the body and put out in another, both processes occurring concurrently during the whole course of the disease.

4. That the hydræmic plethora occurs later in the course of the disease than is suggested by Starling.

5. That the dropsy occurs quite independently of the actual height of the capillary pressure or of the total volume of the blood.

I may here mention that the blood volume of the ascitic dog which I have referred to above was increased. I am aware of one observation only in which the blood volume of a patient with uncompensated heart disease was actually estimated. This was an observation of Lorrain Smith (1902[10]), in which he found an increase in the blood volume.

The blood volume in my experiments was estimated at the different stages in the course of the disease which have been described in Section 3. It has been shown that the maximum error in the estimation of the blood volume of the normal cats by the method employed here was 8 c.c. This amount was, therefore, allowed for in calculating the increase or diminution of blood volume above or below that which was calculated as one twenty-fifth of the body weight in grms. expressed in c.c. The results obtained, therefore, represent values which are as accurate as can be obtained by this method and certainly are not above the actual blood volumes.

The following experiments were performed :—

FIRST WEEK.

Experiment 1.—CAT (weight, 2805 grms.).—Inferior vena cava constricted to a diameter of 3 mm. Blood volume, twenty-four hours later = 120·1 c.c. Ascites present = 33 c.c.

Experiment 2.—CAT (weight, 2640 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, twenty-four hours later = 121·8 c.c. Ascites present = 10 c.c.

Experiment 3.—CAT (weight, 3350 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, twenty-four hours later = 110 c.c. Ascites present = 72 c.c.

Experiment 4.—CAT (weight, 2595 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, forty-eight hours later = 90 c.c. Ascites present = 40 c.c.

Experiment 5.—CAT (weight, 3420 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, forty-eight hours later = 120 c.c. Ascites present = 40 c.c.

Experiment 6.—CAT (weight, 3170 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, forty-eight hours later = 124 c.c. Ascites present = 27 c.c.

Experiment 7.—CAT (weight, 3340 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, three days later = 130 c.c. Ascites present = 10 c.c.

Experiment 8.—CAT (weight, 2910 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, three days later = 128·2 c.c. Ascites present = 84 c.c.

Experiment 9.—Cat (weight, 3400 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, five days later = 133·3 c.c. Ascites present = 30 c.c.

Experiment 10.—Cat (weight, 3753 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, six days later = 145·2 c.c. Ascites present = 15 c.c.

In the first three experiments the animals were killed at the end of twenty-four hours. The blood volume of the first was normal, of the second increased by 7 c.c., and of the third decreased by 16 c.c.

Of those killed at the end of forty-eight hours the blood volume of the first was decreased by 6 c.c., of the second decreased by 9 c.c., and of the third was normal.

The blood volume of the first animal which had lived three days was normal, and of the second increased by 4 c.c.

The blood volumes of the two animals which had lived five and six days respectively were normal.

During the first week, therefore, there was a very slight increase in two cases, a diminution in three, and in five cases the blood volume was normal.

Second Week.

Experiment 11.—Cat (weight, 3360 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, eight days later = 116·5 c.c. Ascites present = 100 c.c.

Experiment 12.—Cat (weight, 2300 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, nine days later = 128·4 c.c. Ascites present = 4 c.c.

Experiment 13.—Cat (weight, 2920 grms.)—Inferior vena cava constricted to 3 mm. Blood volume, fourteen days later = 140 c.c. Ascites present = 80 c.c.

Experiment 14.—Cat (weight, 3120 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, fourteen days later = 139·5 c.c. Ascites present = 12 c.c.

The animal killed on the eighth day showed a diminution in its blood volume of 10 c.c. The other three animals showed an increase of 28 c.c., 15 c.c., and 6 c.c. respectively.

Third Week.

Experiment 15.—Cat (weight, 3038 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of the third week = 140 c.c. Ascites present = 5 c.c.

Fourth Week.

Experiment 16.—Cat (weight, 2680 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of the fourth week = 144·6 c.c. Ascites present = 100 c.c.

Fifth Week.

Experiment 17.—Cat (weight, 2980 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of four weeks and four days = 150·6 c.c. Ascites present = 90 c.c.

Experiment 18.—Cat (weight, 3060 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of five weeks = 148·7 c.c. Ascites present = a trace.

Experiment 19.—Cat (weight, 2550 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of five weeks = 156·4 c.c. Ascites present = 150 c.c.

The blood volume of each animal in the third, fourth, and fifth weeks was increased. It was increased in the third week animal by 9 c.c., and in the fourth week animal by 29 c.c. Of the three animals in the fifth week it was increased in the first by 23 c.c., in the second by 18 c.c., and in the third by 46 c.c.

Seventh Week.

Experiment 20.—Cat (weight, 3850 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of seven weeks = 191·3 c.c. Ascites present = a trace.

Eighth Week.

Experiment 21.—Cat (weight, 3550 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of eight weeks = 197 c.c. Ascites present = 12 c.c.

Eleventh Week.

Experiment 22.—Cat (weight, 3455 grms.).—Inferior vena cava constricted to 3 mm. At the end of three weeks a fair amount of ascites was present. Blood volume, at the end of eleven weeks = 160·7 c.c. No ascites present.

Thirteenth Week.

Experiment 23.—Cat (weight, 4025 grms.).—Inferior vena cava constricted to 3 mm. On the third day after the operation ascites was present, and at the end of seven weeks only a trace was left. Blood volume, at the end of thirteen weeks = 176 c.c. No ascites present.

Seventeenth Week.

Experiment 24.—Cat (weight, 3690 grms.).—Inferior vena cava constricted to 3 mm. At the end of fourteen days ascites was found to be present. Blood volume, at the end of four months and four days = 171 c.c. No ascites present.

Eighteenth Week.

Experiment 25.—Cat (weight, 3410 grms.).—Inferior vena cava constricted to 3 mm. Blood volume, at the end of seventeen weeks and two days = 181·3 c.c. Ascites present = 15 c.c.

In all the above experiments from the seventh week onwards the blood volume was increased. The six animals showed increases of 29 c.c., 47 c.c., 14 c.c., 7 c.c., 16 c.c., and 37 c.c. respectively.

The general statements are justifiable, that during the first week the blood volume of the dropsical animal is normal in half the cases; that it is diminished in the majority of the remainder and in the minority slightly increased; and that after this period it begins to increase and remains high after the disappearance of the dropsy.

Whether the blood volume becomes normal again at a future period of the animal's existence is not decided by these experiments. Probably it does not fall to the normal limit, because the capacity of the vascular system is increased. The vessels of the congested area are dilated, as may be seen in sections of the liver in which the dilatation goes on to the formation of sinuses; the anastomotic veins are also very large. Naturally, then, if this state of affairs is permanent the volume of the blood will remain increased owing to increased capacity of the containing system.

During the first two weeks the volume of the blood bears no relation to the amount of the ascites. It will be seen on reference to the previous section that the output of lymph into the peritoneum is very active from the first day and that the fluid accumulates in large quantities during the first week. At this precise period the volume of the blood is usually normal or diminished. The amount of ascites in the first twelve experiments quoted is particularly large when the blood volume is small, and *vice versa*. There is only one exception to this statement, namely, Experiment 8, and in this experiment the blood volume was only increased by 4 c.c.

The numbers are again quoted in illustration of this statement (Table V.).

TABLE V.

Experiment.	Time.	Blood Volume.	Ascites.
1 . . .	1 day	Normal	33 c.c.
2 . . .	1 ,,	Increase of 7 c.c.	10 ,,
3 . . .	1 ,,	Decrease of 16 ,,	72 ,,
4 . . .	2 days	,, 6 ,,	40 ,,
5 . . .	2 ,,	,, 9 ,,	10 ,,
6 . . .	2 ,,	Normal	27 ,,
7 . . .	3 ,,	,,	10 ,,
8 . . .	3 ,,	Increase of 4 c.c.	34 ,,
9 . . .	5 ,,	Normal	30 ,,
10 . . .	6 ,,	,,	15 ,,
11 . . .	8 ,,	Decrease of 10 c.c.	100 ,,
12 . . .	9 ,,	Increase of 28 ,,	4 ,,

The increase in the blood volume can in no way, therefore, be said to be the cause of the dropsy, since the primary and essential cause or causes is or are acting during the first twenty-four hours, and later before there is any increase in the blood volume. It appears that when the ascites is profuse the volume of the blood is diminished owing to loss of fluid from it. If the increase in blood volume is, therefore, a secondary phenomenon and not the primary and essential cause of the dropsy, it may yet be a contributing factor, since when the blood volume is definitely increased and the first two weeks are passed the amount of the ascites varies with it, roughly, both when the ascites is at its height and when it is subsiding.

A reference to Experiments 13 to 25 illustrates these facts. Experiments 15 and 18 are the only two exceptions.

In Experiments 13 to 19 the dropsy is increasing. The numbers are again quoted (Table VI.).

TABLE VI.

Experiment.	Time.	Blood Volume.	Ascites.
14 . . .	2 weeks	Increase of 6 c.c.	12 c.c.
15 . . .	3 ,,	,, 9 ,,	5 ,,
13 . . .	2 ,,	,, 15 ,,	80 ,,
18 . . .	5 ,,	,, 18 ,,	Trace
17 . . .	4 ,, & 4 days	,, 23 ,,	90 c.c.
16 . . .	4 ,,	,, 29 ,,	100 ,,
19 . . .	5 ,,	,, 46 ,,	150 ,,

When the dropsy is subsiding the same rule obtains, as illustrated by Experiments 20 to 25 (Table VII.).

TABLE VII.

Experiment.	Time.	Blood Volume.	Ascites.
23 . . .	13 weeks	Increase of 7 c.c.	Nil
22 . . .	11 ,,	,, 14 ,,	,,
24 . . .	16 ,, & 4 days	,, 16 ,,	,,
20 . . .	7 ,,	,, 29 ,,	Trace
25 . . .	17 ,, & 2 days	,, 37 ,,	15 c.c.
21 . . .	8 ,,	,, 47 ,,	12 ,,

If the increased blood volume is a contributing cause and thus increases the existing dropsy, it cannot be a very important one, because in spite of its existence the dropsy slowly vanishes. One must, however, remember that at this stage the portal system is becoming relieved by the establishment of anastomoses.

In the early stages, we have seen that the abdominal vessels distend with blood and, since the volume of the blood is strictly limited, the other portions of the vascular system are depleted. This is particularly the case since the accumulation of ascites by abstracting fluid from the abdominal vessels may actually diminish the total volume of the blood. Absorption naturally occurs rapidly in the depleted areas, and the connective tissue in these regions becomes excessively dry.

One has, therefore, absorption in the depleted areas and output from the abdominal vessels. If the one balances the other the total volume of blood remains normal, if output overbalances absorption the blood volume will diminish. Both these two conditions actually occur.

As the ascites collects in greater quantity, the flow of lymph through the thoracic duct increases, and more and more lymph is returned to the venous system. The efforts of the animal are thus directed towards increasing its blood volume, but the increase is retarded by the output of increasing ascites. The continual absorption in the depleted areas and the return of the ascitic fluid to the blood, however, leads finally to an increase of the blood volume varying in degree and time of onset in each case. This is rendered possible by the retention in the body of fluid, owing to deficient excretory action of the kidneys.

The reason the excess of fluid is not finally got rid of is that, as already stated, the capacity of the vascular system is increased. The condition here is not, therefore, analogous to the artificial production of plethora by injection of blood or saline solution into the normal animal.

6. *Hæmoglobin Content of the Blood in the Dropsical Animal.*

During the past twenty years or more many observations, of which the following are the most important, have been made upon the condition of the blood in uncompensated heart disease, particularly with regard to its content of water and hæmoglobin.

The methods employed were either that of weighing the dried residue of the blood, of measuring its specific gravity, or of ascertaining the percentages of hæmoglobin and red corpuscles which it contained.

Hammerschlag (1892[11]) found that in many cases of cardiac insufficiency there was a slightly higher percentage of water in the blood than normal, but in some cases the concentration was normal.

Stintzing and Gumprecht (1894[12]) found hydræmia in several cases, and also that as the œdema subsided and compensation was restored, the percentage of water in the hydræmic blood diminished; also, conversely, that the blood became diluted at the onset of dropsy. They considered that the blood volume was increased.

Grawitz (1895[13]) explained the dilution of the blood, which he observed in uncompensated heart disease, on the supposition that the fall of arterial pressure which occurred, and the consequent relaxation of the smallest vessels, caused a transudation of tissue fluid into them.

Askanazy (1897 [14]) drew from his observations the conclusions that the blood serum was always more or less diluted, and that in general the dilution varied in proportion to the degree of dropsy, that it increased as the dropsy increased, and diminished and finally disappeared when compensation was restored.

Lakin (1910 [15]) investigated twenty-five cases of cardiac œdema. In ten the hæmoglobin percentage was normal, in four it was above normal, and in eleven it was below normal. He also found in four of the above cases a temporary rise in the hæmoglobin percentage while the œdema was subsiding, the percentage finally returning to normal.

The hæmoglobin estimations to be now described explain how it is that the blood in uncompensated heart disease may be either of normal concentration or above or below this level.

On some day during the first week the percentage of hæmoglobin in the blood begins to decrease. This never occurs on the first day after the operation, because every case examined at the end of twenty-four hours showed a normal or slightly increased percentage. It may commence on the second day, but only in about one out of six cases. Two-thirds of the cases show a commencing fall of the hæmoglobin percentage on the third day, and the remainder on the fourth, fifth, or sixth days. One case showed a normal percentage on the eighth day. With this last exception every case out of seventy-six estimations, conducted from the seventh day to the end of the fifth week, showed a decrease in the hæmoglobin percentage.

After this period, although the blood volume remains increased, the hæmoglobin percentage rises, becoming normal or slightly above this level in most of the cases during the next few weeks. Of five cases examined after three months, two showed decreases of 6 and 8 per cent. respectively, one was normal, and the remaining two were increased by 6 and 14 per cent. respectively. One hundred and seventy estimations in all were made on forty animals.

The average decreases were observed at the stages mentioned (Table VIII.).

TABLE VIII.

Time.	Average Decrease in Hb Percentage.	Maximum.
1st week	13	25
2nd ,, ...	16·6	32
3rd ,, ...	19·3	40
4th ,, ...	19·4	36
5th ,, ...	15·1	28
6th ,, ...	10·1	24
7th ,, ...	10·4	18

After this period only about four or six estimations a week were made, so the averages were not reliable enough to quote.

The following table shows the relation of the blood volume, the hæmoglobin percentage, and the amount of ascites in the twenty-five experiments quoted above.

The hæmoglobin was estimated in each normal animal before the operation, and subsequently at intervals during its life, and finally on the day the blood volume was ascertained (Table IX.).

TABLE IX.

Experiment.	Time.	Blood Volume.	Hb percentage.	Ascites.
1 . .	1 day	Normal	Normal	33 c.c.
2 . .	1 ,,	Increase of 7 c.c.	,,	10 ,,
3 . .	1 ,,	Decrease of 16 ,,	,,	72 ,,
4 . .	2 days	,, 6 ,,	,,	40 ,,
5 . .	2 ,,	,, 9 ,,	,,	40 ,,
6 . .	2 ,,	Normal	,,	27 ,,
7 . .	3 ,,	,,	Decrease of 25 per cent.	10 ,,
8 . .	3 ,,	Increase of 4 c.c.	Increase of 20 ,,	84 ,,
9 . .	5 ,,	Normal	,, 6 ,,	30 ,,
10 . .	6 ,,	,,	Decrease of 20 ,,	15 ,,
11 . .	8 ,,	Decrease of 10 c.c.	Normal	100 ,,
12 . .	9 ,,	Increase of 28 ,,	Decrease of 12 per cent.	4 ,,
13 . .	2 weeks	,, 15 ,,	,, 10 ,,	80 ,,
14 . .	2 ,,	,, 6 ,,	,, 26 ,,	12 ,,
15 . .	3 ,,	,, 9 ,,	,, 22 ,,	5 ,,
16 . .	4 ,,	,, 29 ,,	,, 10 ,,	100 ,,
17 . .	4½ ,,	,, 23 ,,	,, 16 ,,	90 ,,
18 . .	5 ,,	,, 18 ,,	,, 6 ,,	Trace
19 . .	5 ,,	,, 46 ,,	,, 24 ,,	150 c.c.
20 . .	7 ,,	,, 29 ,,	Normal	Trace
21 . .	8 ,,	,, 47 ,,	,,	12 c.c.
22 . .	11 ,,	,, 14 ,,	Decrease of 10 per cent.	Nil
23 . .	13 ,,	,, 7 ,,	,, 6 ,,	,,
24 . .	16 ,,	,, 16 ,,	Increase of 6 ,,	,,
25 . .	17 ,,	,, 37 ,,	Normal	15 c.c.

We see from the above table that the period of continuous increase in the blood volume corresponds with the period of continuous decrease in the hæmoglobin percentage, and that after this, although the blood remains increased in volume, the hæmoglobin percentage rises.

It appears that the decrease in the hæmoglobin content of the blood is due to a dilution of the blood, which increases its volume, and that the subsequent increase in the hæmoglobin content is an effort on the part of the animal to restore its hæmoglobin percentage to the normal limit. It may succeed in doing this whilst ascites is present, or after it has disappeared. This effort may be the result of a diminution in the oxygen tension of the blood acting as a stimulant to the red bone-marrow.

The degree of reduction of the hæmoglobin percentage does not appear to bear any definite relation to the quantity of ascites present.

Some of the cases in which the hæmoglobin was repeatedly estimated are now quoted in order to show that sometimes it decreases and subsequently increases quite regularly, and that at other times variations occur (Table X.).

TABLE X.

Experiment.	Normal p. cent. of Hb.	Day.	Hb.	Day.	Hb.	Day.	Hb.	Day.	Hb.	Day.	Hb.	Day.	Hb.	Day.	Hb.	Day.	Hb.
14	86	3	78	10	74	14	60	...		...		...		...		...	
15	86	3	70	7	70	14	62	21	64	...		...		...		...	
16	78	4	76	6	76	15	72	21	76	28	68	...		...		...	
17	84	3	72	10	60	14	62	18	64	22	72	29	76	32	68	...	
18	80	6	68	10	70	14	68	18	74	25	76	35	74	...		...	
19	80	1	92	3	80	4	66	19	62	25	62	29	60	33	52	35	52
		36	56	...		...		...		...		...		...		...	
20	86	5	80	11	74	14	76	18	62	24	52	28	50	32	70	38	78
		43	68	48	86	...		...		...		...		...		...	
21	86	6	86	10	80	17	68	19	68	22	72	26	68	30	72	33	76
		36	80	42	84	47	84	52	86	...		...		...		...	
22	84	2	76	6	80	13	70	17	76	24	72	26	72	29	78	33	76
		37	72	40	74	43	66	49	76	54	76	59	76	65	74	75	74
23	88	3	74	7	66	15	66	17	60	19	72	22	68	25	64	29	60
		34	62	40	64	46	76	50	68	60	66	67	70	73	72	81	80
		87	82	...		...		...		...		...		...		...	
24	82	6	68	16	60	25	60	32	64	47	66	60	70	104	88	...	
25	80	10	74	20	70	29	70	38	74	53	74	66	78	108	80	...	
31	78	6	66	13	66	...		...		...		...		...		...	
32	84	7	64	16	44	21	54	...		...		...		...		...	
33	82	7	68	16	56	28	56	...		...		...		...		...	
34	88	5	76	14	56	23	58	35	70	...		...		...		...	
35	82	5	70	14	62	23	56	38	70	41	78	...		...		...	
36	82	6	90	16	70	24	74	35	74	44	76	57	84	...		...	
40	88	7	80	17	60	25	66	36	72	45	76	60	76	70	80	...	
41	100	3	92	6	80	12	70	15	70	...		...		...		...	
43	72	1	72	3	66	5	60	6	50	11	56	32	68	38	78	42	74
		46	74	...		...		...		...		...		...		...	

The reduction or increase in the percentage of hæmoglobin is coincident with, and dependent upon, a reduction or increase in the percentage of red corpuscles.

Two experiments have already been quoted in a former paper (1909 [2]) which show that twenty-four hours after the operation for constriction of the inferior vena cava, in which ascites results, if the percentage of hæmoglobin remains normal that of the red corpuscles likewise remains normal; and if the percentage of hæmoglobin is increased, that of the red corpuscles is also increased.

The following two experiments show that a constant reduction in hæmoglobin percentage is associated with a constant reduction in the corpuscular percentage.

Experiment 43.—Cat (weight, 2930 grms.).—Hæmoglobin = 72 per cent. Red corpuscles = 6,160,000 per c.mm. Inferior vena cava constricted to 3 mm.

On the third day . . . Hb = 66 per cent.
,, fifth day . . . Hb = 60 ,,
,, sixth day . . . Hb = 50 ,,
Red corpuscles = 5,264,000.
(Considerable amount of ascites present.)

Experiment 20.—Cat (weight, 3850 grms.).—Hb = 86 per cent. Red corpuscles = 11,152,000. Inferior vena cava constricted to 3 mm.

On the fifth day . . .	Hb = 80 per cent.
,, eleventh day . .	Hb = 74 ,,
,, fourteenth day . .	Hb = 76 ,, Red corpuscles = 8,160,000 per c.mm.
,, eighteenth day . .	Hb = 62 per cent. Red corpuscles = 5,280,000 per c.mm.

(Abdomen much swollen with a large amount of ascites.)

,, twenty-eighth day .	Hb = 50 per cent. Red corpuscles = 4,912,000 per c.mm.

Finally, it was thought advisable to find out whether the hæmoglobin percentage was the same, whether the blood was drawn from the depleted or the congested area.

The following experiments show that the concentration of the blood is the same in all the vascular areas of the body.

Experiment 16.—Cat (weight, 2680 grms).—Hb (blood of ear) = 78 per cent. Inferior vena cava constricted to 3 mm.

On the fourth day . . .	Hb (blood of ear) = 76 per cent. Hb (,, foot) = 76 ,,
,, sixth day . . .	Hb (,, abdominal wall) = 78 per cent.

(Considerable amount of fluid in abdomen.)

,, twenty-first day . .	Hb (blood of ear) = 76 per cent. Hb (,, abdominal wall) = 76 per cent.

Experiment 42.—Cat (weight, 3800 grms.).—Hb (blood of ear) = 86 per cent. Inferior vena cava constricted to 3 mm.

On the second day . . .	Hb (blood of ear) = 106 per cent. Hb (,, abdominal wall) = 106 per cent. Hb (blood of liver) = 110 per cent.

(Ascites 25 c.c. present.)

Experiment 23.—Cat (weight, 4025 grms.).—Hb (blood of ear) = 88 per cent. Inferior vena cava constricted to 3 mm.

On the third day . . .	Hb (blood of ear) = 74 per cent.
,, seventh day . .	Hb (,, ,,) = 66 ,, Hb (,, abdominal wall) = 64 per cent.

(Ascites present.)

Experiment 41.—Cat (weight, 3500 grms.).—Hb (blood of ear) = 100 per cent. Inferior vena cava constricted to 3 mm.

On the third day . . .	Hb (blood of ear) = 92 per cent.
,, sixth day . . .	Hb (,, ,,) = 80 ,,
,, twelfth day . . .	Hb (,, ,,) = 70 ,,
,, fifteenth day . .	Hb (,, ,,) = 70 ,, Hb (,, liver) = 74 per cent.

(Considerable amount of ascites present.)

7. *Vascular Pressures of the Dropsical Animal.*

When my former experiments, in which it was shown that the preliminary fall of arterial pressure was recovered from in the animal, were performed, there were no clinical observations to show what the height of the arterial pressure was in cardiac dropsy in the human subject.

When the present instrument for registering the human blood pressure came into use, it was soon found that the arterial pressure was normal in cardiac dropsy, thus establishing in the case of the human subject the fact which I had shown experimentally. The first series of observations directly bearing on this point was published by H. J. Starling (1906 [16]). He showed that in cardiac dropsy the arterial pressure was normal, and that only just before death occurred did a fall of pressure take place. In another group of cases he found the arterial pressure above normal, the latter group containing cases of arterial and kidney disease.

The vascular pressures were measured in thirty-four animals. The pressure at the lower end of the inferior vena cava was above normal in all the cases except four. The pressure was on the whole higher after the first week, when the volume of the blood was increased. I have already shown that the venous pressure after the preliminary fall rose again to the height it stood at immediately after the inferior vena cava was constricted. This rise is due to vasomotor constriction, and corresponds with a similar rise in the arterial pressure.

When the blood increases in volume, the venous pressure rises higher still, owing to the excessive filling of the veins.

The height of the venous pressure, therefore, in the first instance, depends upon the degree of vasomotor tone, and it is added to by the subsequent increase in volume of the blood. Although the venous pressure is on the whole highest when the blood is increased in volume, there is great variation in the relation between the actual pressure and the degree of increase of the blood volume in individual cases.

The arterial pressure also shows in some cases higher readings when the blood is increased in volume, but in all cases the pressure is within normal limits.

The height of the venous pressure bears no relation to the amount of ascites present. Large amounts of ascites may occur with normal or moderately raised venous pressures, and high pressures are recorded after the ascites has disappeared.

Table XI. shows the blood volumes and amounts of ascitic fluid in the experiments already quoted in relation to the venous and arterial pressures.

The last three cases, together with Experiments 4, 11, 22, 23, and 24, illustrate remarkably well that the venous pressure and the dropsy are quite independent of each other.

TABLE XI.

Experiment.	Time.	Inferior Cava Pressure.	Carotid Pressure.	Blood Volume.	Ascites.
1	1 day	145 mm. $MgSO_4$	130 mm. Hg	Normal	33 c.c.
2	1 ,,	125 ,, ,,	130 ,, ,,	Increase of 7 c.c.	10 ,,
3	1 ,,	145 ,, ,,	100 ,, ,,	Decrease of 16 c.c.	72 ,,
4	2 days	100 ,, ,,	50 ,, ,,	,, 6 ,,	40 ,,
6	2 ,,	135 ,, ,,	90 ,, ,,	Normal	27 ,,
7	3 ,,	150 ,, ,,	130 ,, ,,	,,	10 ,,
8	3 ,,	177 ,, ,,	110 ,, ,,	Decrease of 4 c.c.	84 ,,
10	6 ,,	150 ,, ,,	100 ,, ,,	Normal	15 ,,
11	8 ,,	80 ,, ,,	90 ,, ,,	Decrease of 10 c.c.	100 ,,
12	9 ,,	135 ,, ,,	130 ,, ,,	Increase of 28 ,,	4 ,,
13	2 weeks	180 ,, ,,	130 ,, ,,	,, 15 ,,	80 ,,
14	2 ,,	285 ,, ,,	130 ,, ,,	,, 6 ,,	12 ,,
15	3 ,,	245 ,, ,,	160 ,, ,,	,, 9 ,,	5 ,,
16	4 ,,	190 ,, ,,	100 ,, ,,	,, 29 ,,	100 ,,
17	4 ,, 4 d'ys	260 ,, ,,	130 ,, ,,	,, 23 ,,	90 ,,
18	5 ,,	210 ,, ,,	160 ,, ,,	,, 18 ,,	Trace
21	8 ,,	225 ,, ,,	140 ,, ,,	,, 47 ,,	12 c.c.
22	11 ,,	155 ,, ,,	180 ,, ,,	,, 14 ,,	Nil
23	13 ,,	170 ,, ,,	170 ,, ,,	,, 7 ,,	,,
24	16 ,, 4 d'ys	180 ,, ,,	150 ,, ,,	,, 16 ,,	,,
25	17 ,, 2 ,,	180 ,, ,,	140 ,, ,,	,, 37 ,,	15 c.c.
In the following experiments the blood volume was not taken :—					
26	1 day	80 mm. $MgSO_4$	70 mm. Hg	...	35 c.c.
27	2 days	95 ,, ,,	80 ,, ,,	...	20 ,,
28	11 weeks	235 ,, ,,	160 ,, ,,	...	Nil

In Experiments 4, 11, 26, and 27 the venous pressures are normal, and yet considerable amounts of ascites are present; in Experiments 22, 23, 24, and 28 the venous pressures are high, and yet the ascites which had been present in the early stages of the disease had disappeared.

In Experiments 29–38 (Table XII.) the venous pressures were taken in all parts of the body at various stages of the disease. It will be seen from this table that the pressure in the portal vein follows the pressure in the inferior vena cava, and is raised to about the same proportionate extent. In some cases it appears to be raised to a rather less extent, but one must remember that, although the portal pressure is in the normal animal usually a little higher than the inferior cava pressure, this is not always the case. There is no correspondence between the pressure in the portal vein and the amount of ascitic fluid.

The venous pressures in the feet and head present the same interesting features which I pointed out in a former paper, dealing with the pressures during the first twenty-four hours after the obstruction was produced.

In all these cases, whatever the stage of the disease, the venous pressure in the hind-feet is raised, but it is raised to a less relative extent than is the pressure in the inferior vena cava. The jugular

TABLE XII.

Experiment.	Time.	Carotid.	Inferior Cava.	Portal Vein.	Foot.	Jugular.	Hb.	Ascites.
29	3 days	100 mm. Hg	160 mm. $MgSO_4$	175 mm. $MgSO_4$	185 mm. $MgSO_4$	70 mm. $MgSO_4$	Increase of 6 per cent.	60 c.c.
30	7 ,,	160 ,,	140 ,, ,,	165 ,, ,,	220 ,, ,,	40 ,, ,,	Decrease of 22 ,,	3 ,,
31	2 weeks	140 ,,	215 ,, ,,	205 ,, ,,	240 ,, ,,	55 ,, ,,	,, 12 ,,	20 + ,,
32	3 ,,	140 ,,	150 ,, ,,	175 ,, ,,	200 ,, ,,	40 ,, ,,	,, 30 ,,	5 ,,
33	4 ,,	140 ,,	180 ,, ,,	160 ,, ,,	205 ,, ,,	40 ,, ,,	,, 26 ,,	40 ,,
34	5 ,,	160 ,,	200 ,, ,,	205 ,, ,,	210 ,, ,,	40 ,, ,,	,, 18 ,,	10 ,,
35	6 ,,	170 ,,	215 ,, ,,	200 ,, ,,	275 ,, ,,	70 ,, ,,	,, 4 ,,	Trace
36	8 ,,	165 ,,	180 ,, ,,	170 ,, ,,	230 ,, ,,	65 ,, ,,	Normal	3 c.c.
37	16 ,,	150 ,,	180 ,, ,,	130 ,, ,,	225 ,, ,,	45 ,, ,,	Increase of 6 per cent.	Nil
38	17 ,,	140 ,,	180 ,, ,,	150 ,, ,,	220 ,, ,,	70 ,, ,,	Normal	15 c.c.

venous pressure is in all cases below the normal, as it was immediately after the inferior vena cava was obstructed. Table XIII. illustrates these points, and it will be noted that Experiment 30 is the only one in which the pressure in the feet was increased by more than that in the inferior vena cava.

TABLE XIII.

Experiment.	Inferior Vena Cava.	Feet.	Jugular Vein.
29	Increase of 63 mm. MgSO$_4$	Increase of 25 mm. MgSO$_4$	Decrease of 9 mm. MgSO$_4$
30	,, 43 ,, ,,	,, 60 ,, ,,	,, 39 ,, ,,
31	,, 118 ,, ,,	,, 80 ,, ,,	,, 24 ,, ,,
32	,, 53 ,, ,,	,, 40 ,, ,,	,, 39 ,, ,,
33	,, 83 ,, ,,	,, 45 ,, ,,	,, 39 ,, ,,
34	,, 103 ,, ,,	,, 50 ,, ,,	,, 39 ,, ,,
35	,, 118 ,, ,,	,, 115 ,, ,,	,, 9 ,, ,,
36	,, 83 ,, ,,	,, 70 ,, ,,	,, 14 ,, ,,
37	,, 83 ,, ,,	,, 65 ,, ,,	,, 34 ,, ,,
38	,, 83 ,, ,,	,, 60 ,, ,,	,, 9 ,, ,,

There is, therefore, an alteration in the pressure levels, the venous pressure in the trunk being relatively more raised than that in the hind-limbs. The venous pressure in the head is always below what it was before the constriction was applied. These alterations in the pressure relations, which are precisely what they were before the increase in blood volume commenced, indicate an abstraction of blood from the periphery and its accumulation in the central portion of the circulatory system. The increase in the blood volume is unable to restore the normal pressure relations by increasing the amount of blood in the depleted areas, because that of the congested area becomes further increased also. The primary cause of the increased blood volume is to be found in the efforts of the organism to restore the normal amount of blood to the depleted areas, a task which it appears to be unable to accomplish.

8. *The Intake of Fluid and the Excretion of Urine in the Dropsical Animal.*

The animals were fed on cat's meat and milk. A certain amount of milk was given twice a day, the leavings on each occasion being measured. The urine passed was collected in the usual manner.

The daily amounts of milk consumed and of urine passed were found to vary very considerably in the normal cat. On some days the volume of milk consumed was greater than that of urine passed, and *vice versa.* When the weekly totals were compared it appeared that on the whole the amount of milk consumed was greater than the amount of urine passed. This is, of course, a very rough test, because the animal loses water in other ways, and milk contains solid matter.

The same irregularity in consumption and excretion was found to occur in the dropsical cats, but on the whole fairly definite results with regard to retention or loss of fluid from the body were obtained.

When the weekly totals of milk and urine were compared it was found that in sixteen out of twenty-two cases (about 73 per cent.) there was definite evidence of retention of fluid in the body during the first two to four weeks. After the third or fourth week there was in some a loss of fluid, but in an equal number there was a mixture of gains and losses, or even a gain during the whole life of the animal, but this was only in the minority.

There is no doubt, however, that in the majority of the cases there was retention of fluid in the body during the period of œdema formation and when the blood was becoming dilute and increasing in volume. It would be tedious and unnecessary to give all the details relating to the cases, but one or two examples may be quoted (Table XIV.).

TABLE XIV.

Experiment 24.

Time.	Hb percentage (Normal = 82).	Retention or Loss of Fluid.	Blood Volume.	Ascites.
1st week . .	68	Retention	...	...
2nd ,, . . .	...	,,	...	Present
3rd ,, . .	60	,,	...	...
4th ,, . .	60	Loss	...	...
5th ,, . .	64	,,	...	...
6th ,, . .	...	,,	...	...
7th ,, . .	66	Retention	...	...
8th ,, . .	...	Loss	...	...
9th ,, . .	70	,,	...	...
10th ,, . .	...	,,	...	...
11th ,, . .	...	,,	...	...
12th ,, . .	...	,,	...	...
13th ,, . .	...	,,	...	...
14th ,, . .	...	,,	...	...
15th ,, . .	...	Retention	...	...
16th ,, . .	88	,,	Increase of 16 c.c.	Nil

Table XIV.—*continued.*

Time.	Hb percentage (Normal = 82).	Retention or Loss of Fluid.	Blood Volume.	Ascites.
		Experiment 35.		
1st week . .	70	Retention	...	...
2nd ,, . .	62	,,	...	...
3rd ,, . .	56	Loss	...	Fair amount
4th ,, . .	...	,,	...	...
5th ,, . .	70	Retention	...	...
6th ,, . .	71	Loss	...	Slight
		Experiment 36.		
	Hb percentage (Normal = 82).			
1st week . .	90	Retention	...	...
2nd ,, . .	...	,,	...	Large amount
3rd ,, . .	70	,,	...	...
4th ,, . .	74	Loss	...	...
5th ,, . .	74	,,	...	...
6th ,, . .	...	,,	...	...
7th ,, . .	76	,,	...	...
8th ,, . .	84	,,	...	3 c.c.
		Experiment 40.		
	Hb percentage (Normal = 88).			
1st week . .	80	Retention	...	...
2nd ,, . .	...	,,	...	Large amount
3rd ,, . .	60	,,	...	...
4th ,, . .	66	,,	...	...
5th ,, . .	72	Loss	...	...
6th ,, . .	76	,,	...	...
7th ,, . .	..	,,	...	...
8th ,, . .	...	,,	...	...
9th ,, . .	76	Retention	...	...
10th ,, . .	80	Loss	...	2 c.c.
		Experiment 19.		
	Hb percentage (Normal = 80).			
1st week . .	66	Retention	...	...
2nd ,, . .	...	,,	...	...
3rd ,, . .	62	Loss	...	...
4th ,, . .	60	Retention	...	...
5th ,, . .	52	,,	Increase of 46 c.c.	45 c.c.
		Experiment 17.		
	Hb percentage (Normal = 84).			
1st week . .	72	Retention	...	...
2nd ,, . .	60	,,	...	...
3rd ,, . .	64	,,	...	...
4th ,, . .	76	,,	...	...
4½ ,, . .	68	,,	Increase of 23 c.c.	90 c.c.

TABLE XIV.—*continued.*

In the minority of cases (six out of twenty-two) the figures showed a loss of fluid from the body during the first week or two, as shown by the following :—

Experiment 34.

Time.	Hb percentage (Normal=88).	Retention or Loss of Fluid.	Blood Volume.	Ascites.
1st week . .	76	Loss	...	...
2nd ,, . .	56	,,	...	...
3rd ,, . .	...	Retention	...	Large amount
4th ,, . .	58	,,	...	...
5th ,, . .	70	Loss	...	10 c.c.

Experiment 18.

	Hb percentage (Normal=80).			
1st week . .	68	Loss	...	..
2nd ,, . .	68	Retention	...	...
3rd ,, . .	74	Loss	...	...
4th ,, . .	76	,,	...	...
5th ,, . .	74	,,	Increase of 18 c.c.	Trace

Taking into account all sources of error, these observations fairly well support the view that there is retention of fluid in the body during the period of œdema formation and increase of water in the blood. The figures do not so well show a loss of fluid during the disappearance of the ascites.

The *amount of urine* passed at the different stages of the diseased condition was very similar in all the cases, which agree in showing a definite decrease in the amount passed during the first few days. An increase then occurs, and the excretion becomes normal, with a tendency towards a further increase as the dropsy is disappearing. The amount of urine passed is definitely related to the amount of fluid consumed, and undoubtedly chiefly depends upon this factor.

All the cases agree in this particular, so that it will be necessary to quote the figures of one case only in illustration (Table XV.).

The animals never drink much for the first few days, and therefore pass a diminished amount of urine, which is further diminished by retention of water in the body. Although the excretory power of the kidneys is somewhat lessened by the passive congestion, they act well in response to an increase in the available amount of fluid presented to them to excrete.

TABLE XV.

Experiment 35.

Time.	Urine.	Milk.	Hb percentage (Normal = 82).	Time.	Urine.	Milk.	Hb percentage (Normal = 82).
1st day	60 c.c.	50 c.c.	...	21st day	135 c.c	130 ,,	...
2nd ,,	50 ,,	85 ,,	...	22nd ,,	120 ,,	120 ,,	...
3rd ,,	40 ,,	80 ,,	...	23rd ,,	135 ,,	140 ,,	56
4th ,,	50 ,,	70 ,,	...	24th ,,	110 ,,	130 ,,	...
5th ,,	40 ,,	50 ,,	70	25th ,,	140 ,,	120 ,,	...
6th ,,	60 ,,	70 ,,	...	26th ,,	165 ,,	140 ,,	...
7th ,,	70 ,,	110 ,,	...	27th ,,	160 ,,	130 ,,	...
8th ,,	120 ,,	130 ,,	...	28th ,,	95 ,,	140 ,,	...
9th ,,	60 ,,	140 ,,	...	29th ,,	155 ,,	150 ,,	...
10th ,,	135 ,,	100 ,,	...	30th ,,	130 ,,	160 ,,	...
11th ,,	110 ,,	110 ,,	...	31st ,,	145 ,,	170 ,,	...
12th ,,	70 ,,	110 ,,	...	32nd ,,	175 ,,	180 ,,	...
13th ,,	140 ,,	110 ,,	...	33rd ,,	220 ,,	160 ,,	...
14th ,,	130 ,,	110 ,,	62	34th ,,	100 ,,	150 ,,	...
15th ,,	100 ,,	130 ,,	...	35th ,,	140 ,,	140 ,,	...
16th ,,	190 ,,	135 ,,	...	36th ,,	195 ,,	190 ,,	...
17th ,,	125 ,,	130 ,,	...	37th ,,	220 ,,	195 ,,	...
18th ,,	140 ,,	110 ,,	...	38th ,,	220 ,,	210 ,,	70
19th ,,	110 ,,	130 ,,	...	40th ,,	175 ,,	170 ,,	...
20th ,,	150 ,,	120 ,,	...	41st ,,	250 ,,	210 ,,	...

9. *The Occurrence of Jaundice.*

Jaundice occurred in three of the animals only, and was therefore due, probably, to some accidental cause. It occurred during the first week in each case. The tissues were generally stained, and also the ascitic fluid. The gall-bladder was perhaps a little enlarged, but bile could be squeezed out of the papilla readily, and the fæces were not light in colour. In one of the cases there was "coffee grounds" material in the stomach and small intestine, which gave the guaiacum and turpentine test for blood.

The only explanation I can offer is, that the condition was one of toxic jaundice resulting from a low grade infection. The animals did not appear to be in any way different from the others, neither did their livers show any difference on microscopical examination. The jaundice which sometimes occurs in uncompensated heart disease may have a similar origin.

10. *Summary and Conclusions.*

In considering the results of these experiments, in conjunction with those which were announced at the beginning of this communication, we see that the increased output of lymph into the peritoneum, giving rise to ascites, is merely a link in a very definite chain of events following obstruction of the inferior vena cava or the occurrence of heart failure.

The primary cause of this sequence of changes in the circulation is to be found in the fact that the portal area, including the liver, is so large and distensible that it is able to abstract a sufficient quantity of blood from the general circulation so as to produce a definite depletion of the latter. The above area is the only one in the body which can produce this result. The superior vena cava may be completely ligatured immediately above the right auricle or the inferior vena cava below the diaphragm, and yet there will be no alteration in the height of the mean blood pressure curve. When, however, the inferior vena cava above the diaphragm is narrowed down to three-fifths of its normal diameter, an immediate fall in the mean arterial blood pressure curve results, and if the vein is completely occluded that curve promptly falls to within a few millimetres of the zero line.

A sudden narrowing of the vein or constriction of the pericardium allows an appreciable interval to elapse before many of the results have time to manifest themselves, so that we are able to study the sequence of events. In the case of a gradual development of heart failure, however, the circulatory changes are crowded together, so that if we were able to study our clinical cases effectively we could not unravel the series of phenomena so as to determine the various mechanisms at work.

When the inferior vena cava is experimentally obstructed above the diaphragm the following sequence of events occurs:—

Stage I.

Accumulation of blood in—(1) Abdominal organs; (2) hinder part of body and hind-limbs.
Rise of venous pressure to smallest venules in these parts.
Capillary pressure in these parts also raised, especially in the liver.
Abstraction of blood from head, chest, and fore-limbs.
Fall of venous and capillary pressure in these parts.
Fall of general arterial blood pressure.

The accumulation of blood behind the obstruction is the cause of the rise of venous pressure, which in its turn, by increasing the velocity of the blood through the obstruction, enables the maximum amount of blood to reach the heart. The venous pressure, however, does not rise till a considerable accumulation of blood has occurred, since the veins and capillaries are not filled to distension; it is necessary to constrict the vein to three-fifths of its diameter before a rise of pressure occurs at all. The rise of venous pressure extends right through the liver to the portal vein, and thence to the fine portal radicles; it also extends to the fine venous radicles of the foot. The capillary pressure in all the parts drained by the inferior vena cava rises. In a former paper I found that this rise was from about 4 to 8 mm. Hg, so that, if the normal capillary pressure were 30 mm. Hg, after obstruction of the vein the pressure would stand at 34 to 38 mm. Hg. We must, however, remember that the height of the capillary pressure depends not only upon the rate at which blood leaves the capillaries, but also upon the rate at which blood is supplied to them. If the arterial pressure remained normal and these vessels contained the normal amount of blood, the capillary pressure after obstruction would stand at the height indicated above, but blood is abstracted from the arteries and the pressure in them falls. Less blood than normal is, therefore, supplied to the capillaries, so that for this reason the pressure within them is not raised to the level indicated by the height of the venous pressure.

Exactly what lessening of effect is produced by the fall of arterial pressure it is impossible to say; we can only conclude that the capillary pressure is raised, but to a much smaller extent than one would expect from the height of the venous pressure. Another point to be taken into consideration is, that in the normal condition many capillaries are more or less empty, and that these capillaries have to be filled out with blood after the obstruction is applied before any rise of capillary pressure could occur. I believe this is one of the reasons why the venous pressure does not rise until the vein is narrowed to a considerable extent. The pressure in the liver capillaries is raised to a greater extent because the pressures in both the hepatic vein and the portal vein are raised.

The abstraction of blood from the head, chest, and upper limbs, together with the fall of general arterial pressure, naturally results in a fall of venous and capillary pressure to these parts.

Stage II.

Dilatation of—(1) Veins and capillaries of abdominal organs and particularly of the liver: (2) inferior vena cava and veins opening directly into it: also of capillaries of retroperitoneal tissue and surrounding parts.
Fall of venous pressure to normal or thereabouts.
Fall of capillary pressure below normal in these parts except in liver when it is normal.
Hind-limbs and posterior part of body wall now contain less blood than normal, like forepart of the body.
Fall of capillary pressure below normal in these parts.
Absorption of lymph in depleted areas of body.
Increased output of lymph in plethoric areas leading to—(1) ascites; (2) œdema of retroperitoneal tissue.

The distensibility of the abdominal vessels is responsible for a further depletion of the forepart of the body, and the hind-limbs now share in this depletion. Owing to this increased capacity of a part of the venous system, the pressures in all the parts of the vascular system naturally fall, the blood volume remaining normal.

The venous pressures in all parts behind the obstruction being normal and the arterial pressure low, it naturally follows that the capillary pressure is subnormal in all parts except the liver, where it is normal. The capillary pressure in the forepart of the body falls lower than it was before.

The increased output of lymph in the plethoric areas giving rise to ascites usually exactly balances the absorption of lymph in the depleted areas, so that the blood is neither diluted nor increased in volume. Occasionally, however, absorption may fall behind and the blood become somewhat concentrated. The increased absorption of lymph is shown by the excessive dryness of the cellular tissue in all the depleted areas.

It is to be noted that the lumbar veins which open directly into the inferior vena cava are dilated and plethoric, and œdema occurs by increased output of lymph from their capillaries (retroperitoneal tissue), whereas the anterior and posterior branches of these veins (abdominal wall) and the veins of the hind-limbs, owing to their much greater distance from the seat of obstruction, actually contain less blood than normal, and increased absorption of lymph takes place into their capillaries.

Stage III.

Vasomotor constriction leading to—(1) Rise of arterial pressure to the normal.

(2) Rise of venous pressure in the central portions of the trunk to its former high level.

(3) Rise of capillary pressure in these parts to normal or above.

(4) Venous pressure in the hind-limbs remains at the normal level.

(5) Capillary pressure in these parts remains subnormal.

The vasomotor constriction automatically occurs by stimulation of the vasomotor centre owing to deficient blood supply to the brain. If it did not occur it would be impossible for the high venous pressure in the trunk to be maintained, since the vascular system contains its normal amount of blood only. The high venous pressure is necessary so as to increase the velocity of blood through the constriction, in order that the maximum amount possible may reach the heart. Neither would it be possible without vasomotor constriction to ensure the maximum supply of blood to the brain.

These alterations in venous and arterial pressures in their turn affect the capillary pressure. The rise of venous pressure results in a rise of capillary pressure in the trunk, but this rise is minimised by the arterial constriction which cuts down the supply of blood to the capillaries, so that it is doubtful if the capillary pressure here rises above its normal level. The venous pressure in the legs remaining at the normal level, the capillary pressure in these parts is subnormal.

The venous pressure levels have, therefore, an altered relation, the pressure in the legs being lower relatively to that in the trunk than in the normal animal.

These alterations in pressure make no difference to the absorption and output of lymph and to the accumulation of dropsical fluid. They occur at various times according to the power of recovery of the animal, and are usually completed within the first twenty-four hours following the application of the constriction.

Stage IV.

Continuation of lymph absorption in depleted areas.
Continuation of lymph output in plethoric areas.
Increasing flow of lymph in thoracic duct as ascites accumulates.
Diminished excretion of urine.
Increased amount of water in the blood and fall in percentage of hæmoglobin.
Condition of hydræmic plethora established.
Venous and capillary pressures raised in plethoric area.
Venous and capillary pressures raised in hind-limbs, but to a less relative extent than in the trunk.
Venous and capillary pressures below normal in the head and forepart of the trunk.

This state of affairs is not recognisable in most cases till the end of the first week. It increases and continues till about the end of the third or fifth week, when it gives place to the next stage. The hydræmic plethora varies very much in amount and is a purely secondary process, depending upon—(1) Continual absorption of lymph from the depleted areas; (2) when the ascites has reached a certain degree, the rapid return to the blood of most of the ascitic fluid which is produced, so that the ascites only slowly accumulates; and (3) retention of a certain amount of fluid in the body owing to diminished excretory activity of the kidneys. It is to be noted that although hydræmic plethora exists, the distribution of the blood in the vascular system is still uneven, the plethoric areas being more markedly plethoric, the hind-limbs containing a little more than their normal amount of blood, and the head and forepart of the body still being depleted, as shown by their low venous pressures. The animal is thus seen to be endeavouring to make up the deficiency of blood in its depleted areas, and it does not succeed in doing this except in the hind-limbs, but it produces an increasing plethora of its plethoric areas. The latter are relieved rapidly by the production of ascites, and the ascitic fluid is quickly returned to the blood, so that a constant and rapid circulation of lymph goes on, the ascites gradually increasing as output overbalances absorption.

This state of things would undoubtedly go from bad to worse were it not for the gradual establishment of anastomoses. These are rapidly formed in the abdominal wall and gradually increase in size; the anastomoses of the portal circulation are, however, much slower in forming. The height of this morbid condition is perhaps at the fourth or fifth week, when the portal anastomoses begin to effect a reduction in the processes described. The output into the peritoneum becomes less and less rapid, the lymph flow in the thoracic duct also falling as a result, but not quite so rapidly, so that the ascites gradually disappears.

Although a secondary process, the hydræmic plethora has two results. It (1) accelerates the output of lymph into the peritoneum, and (2) alters the venous and capillary pressures as described.

Stage V.

The ascites gradually disappears.

The hydræmic plethora becomes a true plethora, the percentage of hæmoglobin gradually returning to the normal.

A true plethora thus becomes established probably because there is an active formation of blood corpuscles owing to stimulation of the red bone-marrow. The reason the blood volume remains high is probably because the total capacity of the vascular system is increased owing to the dilatation of the vessels of the liver, and large veins of the trunk, and the presence of large anastomotic veins. The venous pressures are kept up by the plethora and the altered circulatory conditions.

We thus see that neither plethora nor a high capillary pressure are the primary causes of the increased output of lymph giving rise to the dropsy, because neither is necessary for its production.

The increased venous pressure in the trunk leads directly to enlargement of the liver and dilatation of the other veins, and thus indirectly to abstraction of blood from other parts of the body and increased absorption of lymph in these parts, but the chief *rôle* played by the various alterations in the vascular pressures, arterial and venous, is that of ensuring as ample a circulation of blood through the brain and other organs as is possible under the existing circulatory conditions.

The object of this investigation has been to explain what gross pathological processes are at work in an uncompensated condition of the circulation and the relation of these processes to each other. I do not, therefore, propose to enter into any theoretical considerations with regard to the ultimate factors which are responsible for causing increased absorption of lymph in the depleted areas, and increased output of lymph in the congested areas. One cannot with profit discuss these obscure factors until all the more gross facts relating to the circulatory alterations are found out. These experiments, however, plainly show that purely physical factors, and particularly those which in the past have been held to be direct causes of the dropsy, are not primarily responsible for its presence.

The essential condition for the production of this form of dropsy appears to be one in which an amount of venous blood, above the normal volume, is flowing with a diminished velocity through dilated capillaries at a pressure either normal in height or raised or perhaps even subnormal. In these circumstances there is naturally a diminished absorption of lymph by the capillaries, but this is no doubt merely a subsidiary factor in the production of the dropsy. We know that the circulatory system contains a definite amount of fluid and that any excess above this amount is rapidly got rid of by the secretory and excretory glands and by the capillary walls, the lymph flow in the thoracic duct being greatly exaggerated. What is true of the whole is true of a part, and if any excess of blood in a territory of the vascular system is not rapidly carried off, owing to the increased velocity of the blood in the veins, it is got rid of by the capillary wall. Simple vaso-dilatation in a part increases, enormously, the velocity of flow of blood in the veins, but does not increase the lymph flow, unless an increased functional activity of the part requires more lymph. In the condition we are studying, the excessive amount of blood is retained in the part owing to the diminished velocity of the venous stream, and, moreover, it tends gradually to increase in amount, and the plethoric area becomes more plethoric.

Whether the capillary wall actively gets rid of the excessive amount of fluid, or whether the latter leaks out owing to increased permeability of the capillary wall, caused by injury by metabolic products, must remain a subject for speculation. It is significant that the ascites disappears owing to the output of lymph becoming gradually less and less as the surplus of blood is gradually diverted by the growing anastomoses. It appears as if the diminishing output of lymph was conditioned rather by the increasing ease with which the surplus of blood in the part is carried off, than by a lessening degree of damage to or a slow recovery of the capillary wall. The rate of output of lymph in the congested area varies during the course of the disease, and it is difficult to explain this occurrence by the supposition that corresponding variations occur in the degree of damage done to the capillary wall.

In the condition of uncompensated heart disease the following sequence of events occurs :—

Stage I.

Accumulation of blood in veins.
Rise of venous pressure to smallest vessels in all parts.

Capillary pressure, raised particularly in the liver.
Abstraction of blood from arterial system.
Fall of arterial blood pressure.

Stage II.

Dilatation of—(1) Veins of liver and abdominal organs; (2) large veins of chest and abdomen and their immediate tributaries.
Fall of venous pressure to normal or thereabouts.
Capillary pressure in all parts below normal except in the liver where it is normal.
Veins of the trunk relieved of their accumulated blood to some extent at the expense of those of the legs.
Abstraction of blood from head, arms, and upper part of the trunk (except central portion of chest).
Absorption of lymph in the depleted areas of the body.
Increased output of lymph in the plethoric areas leading to—(1) Ascites, which is usually not very evident till the œdema of the legs is marked; (2) œdema of legs.

Stage III.

Vasomotor constriction leading to—(1) Rise of arterial pressure to the normal.
(2) Rise of venous pressure in central portions of the trunk and the legs to its former high level.
(3) Capillary pressure in these parts returns to the normal or may be raised; it is certainly raised in the liver.
(4) Venous pressure in upper part of body (except centre part of chest) does not rise.
(5) Capillary pressure in these parts subnormal.

Stage IV.

Continuation of lymph absorption in depleted areas.
Continuation of lymph output in plethoric areas.
Increased flow of lymph in the thoracic duct as ascites and dropsy of legs accumulate; the outflow into the veins is somewhat obstructed by the raised venous pressure in the large veins at upper part of chest.
Diminished excretion of urine.
Increased amount of water in the blood and fall in percentage of hæmoglobin.
Condition of hydræmic plethora established.
Venous and capillary pressures further raised in the plethoric areas.

Venous and capillary pressures below normal in the head, arms, and upper part of trunk (except central parts of chest).

Stage V.

The condition tends to progress steadily so that the ascites increases, the œdema spreads up the legs to the abdominal wall and chest, and hydrothorax appears.

The hydræmic plethora gradually develops into a true plethora.

In a slight case of heart failure the normal circulatory conditions are restored before the œdema spreads beyond the feet.

When the patient lies down the œdema of the legs disappears or lessens and the congestion of the viscera is increased, the ascites becoming more evident, and *vice versa*. After repeated attacks of heart failure the capacity of the vascular system is greatly increased, owing to permanent dilatation of the large veins near the heart and enlargement of the liver, and sometimes remarkable dilatation of the heart itself. In such a condition a true plethora exists.

When such a case develops a further attack of dropsy, one sees a characteristic clinical picture, particularly when the patient is sitting in a chair. The patient is water-logged in the lower part of his body; the face, neck, and arms are shrunken from absorption of lymph; the jugular veins are dilated and the face cyanosed. Although there is cyanosis in the upper part of the body owing to slowing of the circulation and deficient supply of oxygen to the tissues, so that the capillary wall should be devitalised, increased absorption, not increased output, of lymph occurs. This is due to the fact that the upper parts of the body actually contain less blood than normal except in the central part of the chest; and it is not uncommon, when bleeding from the arm is attempted in such a case, to find the vein relatively empty of blood and the operation by no means easy to perform.

REFERENCES.

1. C. Bolton . . . . . *Journ. Path. and Bacteriol.*, Cambridge, 1903, vol. ix. p. 67.
2. ,, ... *Proc. Roy. Soc. London* (B), 1907, vol. lxxix. p. 267; *Journ. Path. and Bacteriol.*, Cambridge, 1909, vol. xiv. p. 49.
3. ,, ... *Journ. Path. and Bacteriol.*, Cambridge, 1914, vol. xix. p. 258.
4. C. Bolton and E. H. Starling. *Heart*, 1910, vol. i. p. 292.
5. Andral . . . . . . "Cours de Pathologie Interne," Paris, 1836, tome ii. p. 9.
6. Oertel . . . . . . "Therapie der Kreislaufstörungen," v. Ziemssen's "Handbuch," 1884, Bd. iv.

7. Cohnheim . . . . . "Vorlesungen ueber allg. Pathologie," Berlin, 1882, Aufl. 2, Bd. i.
8. Cohnheim and Lichtheim *Virchow's Archiv*, 1877, Bd. lxix. S. 106.
9. E. H. Starling . . . . "Arris and Gale Lectures," *Lancet*, London, 1896, vol. i. pp. 1267–1407 and 1897, vol. i. pp. 569–723; also "The Fluids of the Body," 1909, pp. 150 and 172.
10. Lorrain Smith . . . *Trans. Path. Soc. London*, 1902, vol. liii. p. 136.
11. Hammerschlag . . . . *Ztschr. f. klin. Med.*, Berlin, 1892, Bd. xxi. S. 475.
12. Stintzing and Gümprecht *Deutsches Arch. f. klin. Med.*, 1894, Bd. liii. S. 265.
13. Grawitz . . . . . . *Ibid.*, 1895, Bd. liv. S. 588.
14. Askanazy . . . . . *Ibid.*, 1897, Bd. lix. S. 385.
15. Lakin . . . . *Med. Chron.*, Manchester, 1910, Ser. IV. vol. xx. p. 5.
16. H. J. Starling . . . . *Lancet*, London, 1906, vol. ii. p. 846.

97.

PATHOLOGICAL CHANGES IN THE ADRENAL GLANDS

By T. R. ELLIOTT[1]

With Plates 5–7

An apology is needed in preface to this paper. The observations that are detailed in it do not lead to any general conclusions of obvious value; but despite that it seemed worth while to place them on record because I believe that they are made with accuracy. Many dogmatic statements on the slightest of evidence have been published with regard to excess of function of this or that part of the adrenal glands. They remain unproven, and even now there is but little to add to the clear and able summary of the subject which was written by Léon Bernard (4) in 1907.

Bernard dealt with the pathological question under three heads:

1. The relationship of the adrenal glands to other glands with internal secretions. Nothing definite, he said, is known of this; and the statement, unfortunately, is still true.

2. The reaction of the glands to infections and intoxications. French workers had observed exhaustion of adrenalin in experimental infections of animals. Bernard pointed out that these results did not make it clear that the adrenals played any essential part in the defence of the organism against infections.

3. In atheroma and hypertension he thought it proved that the renal impermeability was associated with changes in the adrenals in the direction of cortical hyperplasia, but that there was no evidence as to the nature of the lecithinogenic function of the cortex.

To physiologists the healthy gland is known as a threefold complex, of cortex, medulla, and ganglion cells. The cortex is practically a uniform tissue whose cells in the majority of animals are loaded with a special fatty substance, and often in addition with brown pigmented granules at the zone close to the medulla. Arnold's (2) old distinction of the three zones, glomerulosa, &c., is of no real value, and should be set aside. The cortical pigmented substance is a special secretory product, which appears in prodigious quantities in the gland of the guinea-pig. It and its cells may form tumours; but nothing

---

[1] Working in tenure of a Beit Memorial Fellowship, and aided by a grant from the Graham Research Fund, University College Hospital, London University.

[Q. J. M., Oct., 1914.]

is known as to its history, and I have made no special observations with regard to its presence or absence in the pathological glands described in this paper. The medulla stores up adrenalin and excretes it into the blood-stream. Sympathetic ganglion cells occur in small numbers within the medulla of the human gland, and they are always present in masses on its outside, masses which lead up directly to the main semilunar ganglion.

Recent work (10, 17) in the physiological laboratories has abundantly confirmed the first experiments of Dreyer (16) and Tscheboksaroff (44), proving that the medullary chromaffine cells are directly controlled by the splanchnic nerves, so that every strong stimulation of these nerves is attended by an outpouring of adrenalin into the circulation. Indeed, this central core of the gland is very essentially a part of the sympathetic nervous system. Its cells are not equivalent to those of ordinary peripheral glands: they belong to a higher rank, and are practically sympathetic ganglion cells which do not influence the peripheral muscle through the medium of a nervous fibre, but instead reinforce the nervous impulses of the latter by a discharge of adrenalin. Adrenalin circulating in the blood evokes from plain muscle precisely the same response, be it contraction, relaxation, or none at all, as does the sympathetic nervous impulse. Moreover, in the absence of the adrenal glands the plain muscle soon loses tone and fails to respond to the nervous call. Hence it is reasonable to think that the steady tone of all muscles innervated by sympathetic nerves depends not so much on the nervous impulse as on the influence of adrenalin circulating in the blood. To effect this result the chromaffine or 'paraganglion' cells seem to have been differentiated from the mass of the true ganglion cells, and set apart for the task of supplying this special hormone to their own department of the nervous system. The action of each is essential for perfect control: the one to hold the muscle at a uniform level of contraction in its so-called rest, and the other to effect such rapid changes, this way or that, as are needed in the quick reflex activities of the body. The relationship of the two, adrenalin and nervous impulse, may be roughly illustrated by comparing the muscle with the string of a violin, that must be screwed to its proper pitch for the fingers to play upon it and evoke the quickly changing notes.

The muscles that are especially innervated by the sympathetic are of course those of the heart and blood-vessels. They are ever at work, and life must cease if they relax their tension. It is this physiological connexion which gives such deep interest to the study of the glands, and it is this which makes it urgent to gain knowledge of their condition in the various forms of mortal disease.

If we wish, then, to know how bodily disease affects the gland, it will be necessary to consider: (1) Its structural anatomy, changes in gross bulk and in histological detail; (2) alterations in the cortical fatty substance; (3) variations in the load of adrenalin.

The Cortical Lipoid.

In healthy glands all the cells of the cortex are crammed with a curious fatty substance, and the same substance is seen in the vagrant nests of cortical cells that may be embedded in the medulla or ensheath the nerves and large veins entering the medulla. Kaiserling and Orgler (27) first pointed out that this fat is characterized by its doubly refractive properties, and that of all healthy tissues only the adrenal cortex and the cells of the corpus luteum contain anisotropic lipoid, though similar substances occur frequently in the fatty degeneration of slow tissue necrosis. In a review of such fats Aschoff (3) found reason to regard them all as cholesterin esters, with which apparently lecithin[2] is also associated in the adrenals. Tuckett and I (20) described the distribution of this lipoid in various animals in health, and showed its early loss in disease. But most writers in medical pathology have made a mistake arising from the general pathological observation that fat in an organ signifies degenerative processes, and have inferred that the cortical fat is the index of disease or altered functions of the gland. Marchetti (31) examined 1,200 post-mortem cases, believing throughout that the fat was a pathological infiltration; and Napp (34) held a similar view. This opinion is reflected in the chief text-books of medicine, so that, for example, Neusser and Wiesel's (35) monograph (1910) in Nothnagel's *Encyclopaedia* almost disregards the question of the fat. But Loeschcke (30) and also Goldzieher (26) observed that the fat vanishes in all acute infections: and though E. Thomas (43) denied this to be so in children with diphtheria, scarlet fever, or measles, the statement was fully confirmed in Weltmann's (47) elaborate analysis of 600 cases. My own observations were made independently of these to continue in man what I had already seen in animals. The general features of the exhaustion in human glands were referred to in my paper with Armour (19) in 1911, and the details given in the present account agree almost identically with those of Goldzieher and of Weltmann.

Method. The glands were taken from the body as soon as possible after death, and rarely later than ten hours. They were fixed in a mixture of potassium bichromate and formalin, cut on a freezing microtome, and stained with Scharlach R. and Ehrlich's haematoxylin. Sometimes the load of fat was so abundant that the sections could hardly be submerged either in the haematoxylin dye or in water. Controls were always stained with Scharlach alone, so as to correct for loss of red tint if the sections were treated too long with the Ehrlich stain. The sections were then mounted in glycerin, either pure or

---

[2] Bernard found that lecithin formed nearly 7 per cent. of the entire weight of a horse's adrenal, and 50 per cent. of its fat. But Lapworth (29) obtained less than 1 per cent. of cholesterol and its esters in the sheep. The sheep's gland contains practically no fat that can be demonstrated under the microscope. I am not aware of any accurate analyses in the different animals which would compare in each species the amount of lipoid that can be extracted chemically with the fat that is seen by histological methods.

50 per cent., and examined with the polarizing microscope.[3] The doubly refractive crystals seemed to represent a more elaborate condition of the lipoid than that in which it takes a deep stain with Scharlach. Indeed, a gland that is loaded to its utmost capacity in rest shows a field studded with brilliant crystals that are almost pure white or only faintly tinged with pink. It must be remembered that in life the lipoid is fluid, though even then probably anisotropic, and that these permanent crystals are produced by cooling and fixation. As the load is reduced the doubly refractive crystals become smaller and fewer, while the substance stored in the cell takes a deep scarlet stain characteristic of the simpler fats (Pl. 5, Fig. 1). Finally, all vanishes and there remains only a contracted cell of a uniform blue (Fig. 2).

The path by which the lipoid left the cell could not be identified. Fat was never seen in the lymphatics or blood-vessels outside the cortical cells under conditions in which post-mortem autolysis and leakage could be excluded. A cross-section of a cylinder of the cortical cells, when fully loaded, showed the nuclei to be all crowded together into the centre of the column, while the bases of the cells, adjacent to the capillary vessels, were swollen with lipoid. The appearance was unlike what would be expected were secretion to be discharged into some minute lumen within the centre of the column, and it suggested the outward path along the capillaries. Still the centre of the column appeared sometimes to form a potential lumen, for it was noticed to be distended with lymph and even to contain red blood corpuscles in certain cases where thrombosis in the central vein of the medulla had caused considerable rise of pressure in the smaller vessels and so burst a way up the axis of each cylinder[4] (Fig. 2).

The microscopic test, by Scharlach and the polariscope, revealed practically all the lipoid present in the cortex. It has been proved for the liver and the heart that the appearance of fat in their cells may not mean that more fat has been added to the total bulk of these organs, but simply that fat which was always present, but was normally masked in chemical combinations that hid it from the dye, became freed to receive the stain. This was not so in the human adrenals. Dr. F. H. Thiele very kindly made for me a chemical analysis of the fat content of seven glands, and the amounts found corresponded closely with the histological picture.[5] The Scharlach stain is therefore a reliable test for the amount of lipoid in the cortex, though indeed for rough purposes even that is unnecessary. Naked-eye inspection of a cut surface of the fresh gland can at once recognize the lipoid. When abundant, it gives to the tissue a yellow butter-like tint around the greyish-white medulla. In exhaustion the cortex changes from yellow to greyish brown, and is often haemorrhagic.

---

[3] Sections were also stained with Lorrain Smith's Nile Blue sulphate; but this method was soon discarded because the specimens could not be kept, and the distinction between acid and neutral fats, so obtained, did not appear to be of any importance.

[4] Stoerk (42) argued against the existence of such a lumen in the heart of the cell columns, urging that in these haemorrhagic extravasations the blood lay between the cell cylinders and the enveloping connective tissue. I failed in an attempt to inject carmine gelatin into the possible lumen.

[5] Since this was written, an admirable review of the general question by J. W. McNee has appeared in the pages of this Journal. McNee refers to an approaching paper by Landau and himself in which chemical analyses of the cholesterin in the adrenals are fully described and found to accord with the results of microscopic examination.

Apart from nodular outgrowths on the surface of the gland, cortical adenomata were occasionally seen in the medulla, where they might grow to form a considerable mass, as much as 2 cm. in diameter and forming 20 to 40 per cent. of the entire gland's weight. The cells of these were arranged in regular anastomosing columns and contained lipoid like the normal cortex. But the curious feature of the real adenoma was that, despite the full blood supply, its cells parted with their lipoid very slowly and were well laden even in conditions which exhausted the fat of the ordinary cortex (Pl. 6, Fig. 3). No experiments have yet succeeded in demonstrating a nervous control of the fat store in the cortical cells, like that so clearly proved for the adrenalin of the medulla. But the special behaviour of the fat in the adenomata does suggest that there may be some such machinery, and that the lipoid does not disappear simply as the result of certain physico-chemical conditions of the circulating blood.

Adrenalin of Medulla.

The amount of adrenalin present in these cells can be gauged roughly in the smaller animals by the depth of the yellow-brown tint which adrenalin causes them to assume when placed in a solution of chromate salts. This chromaffine reaction is shown very well in the glands of young children, but it is unreliable in adults, so that it is quite fallacious to identify implicitly, as Parkinson (36) and others have done, the depth of stain with the amount of residual adrenalin.

For accurate work the adrenalin must be extracted from the gland, and its amount determined by quantitative methods. Mere qualitative tests, to show the presence of adrenalin in the extract by its causing a rise of blood pressure, are insufficient, because all glands, even those of Addison's disease, contain some adrenalin. For this reason the observations of Mott and Halliburton (33) on the question may be set aside; and indeed their method of dealing with the glands seems to have been in itself faulty, for they found that there was no adrenalin present in what should have been a practically normal gland, a gland taken from a woman liable to mental derangements, but otherwise healthy, who was suddenly choked by food. Quantitative assay of adrenalin in the gland extract has been attempted by several workers who used *colorimetric* methods,[6] chiefly based on Comessatti's (15) pink reaction with mercuric chloride solution. These are in general inaccurate, failing to indicate the full amount of adrenalin.

---

[6] The various colorimetric methods are fully reviewed in Borberg's paper (8 and 9). Borberg himself used the frog's enucleated eyeball, believing it to be a test of great delicacy for minimal quantities of adrenalin; but he did not measure the total amount of adrenalin in the glands of the animals which he studied in various conditions of exhaustion.

Schmorl and Ingier (40) modified the Comessatti method, and their modification increased threefold the amount of adrenalin which the test seemed to indicate. Even so the yield obtained by them as a standard of load in healthy glands was but half of what I found by my method of assay; and Goldzieher's (26) analyses, using Zanfrognini's method by the reduction of manganese peroxide, show a similar deficiency. A more reliable colorimetric method does seem to have been found recently by Folin, Cannon, and Denis (22). But most of my analyses were made before the publication of Folin's method, and in consequence I have adhered throughout to my own more tedious method of intravenous injection into cats.

Method. The glands were removed with the lightest of handling as soon as possible, and often only three or four hours after death; and they were analysed absolutely at once. Dissected clean, they were weighed, and each one well ground in a mortar with sand and 80 to 100 c.c. of neutral Ringer's solution (NaCl 0·6 per cent., KCl 0·025 per cent., $CaCl_2$ 0·025 per cent.). The mixture was rapidly brought to boiling, and filtered through glass wool. The percentage of adrenalin in the extract was determined by comparing its effects after intravenous injection on the blood pressure of a pithed cat with that of a solution of chemically prepared adrenalin of known strength. From the percentage strength was then calculated the total amount of adrenalin present in each gland, in milligrams of the base and not of the chloride.

This comparison can be made with very great accuracy, as I have shown in my paper in the *Journal of Physiology* (17), where the experimental details are given; and the figures obtained by it agree with those of Folin and Cannon to a surprising degree of closeness. The standard solution of 1 : 1,000 adrenalin chloride with a trace of chloroform keeps well for several weeks, and even when diluted to 1 : 40,000, the strength used for injection into cats, it loses only 20 per cent. in standing at room-temperature for two days. The gland extract deteriorates more rapidly, so that it must be analysed without any delay. This loss varies in different cases. For example, one extract (Case 76) fell from 3·2 to 3·0 mg. in 18 hours, a loss of 7 per cent.; while another (Case 82) fell from 1·47 to 1·0 mg., a loss of 30 per cent. in 17 hours. Fresh standard solutions were always used, and the gland extract was invariably injected as soon as it had cooled.

The main sources of error in estimating the total quantity of adrenalin residual in the gland at death must therefore lie in the loss of adrenalin during post-mortem changes of the gland, and in faults in the method of extraction. Cannon believes that a fuller yield can be obtained with an acid solvent instead of neutral Ringer. His method yielded in my hands (18) no better results than my own. Schmorl and Ingier extracted in addition to the gland all the surrounding fat, into which they thought adrenalin transuded, and consequently they were unable to determine the size and weight of the glands. This I did not find to be necessary. Still it must be conceded that the method of extraction used was probably to some extent incomplete, and that the absolute value of the adrenalin load in the gland itself was not fully measured. The analyses were, however, always made under similar conditions, so that the values obtained at different times, and in different glands, can be justly compared with one another.

Post-mortem loss will vary with the temperature of the body and the nature of the autolytic changes, which seem to be different with different forms of death. In cats the right and left glands contain exactly equal amounts of adrenalin, and in these animals it is therefore possible to contrast the accuracy of methods of analysis, and also to determine the exhaustion of adrenalin caused by various factors. I found that an excised cat's gland kept on ice for six hours lost practically no adrenalin, whereas one kept at room-temperature (17° C.) in a moist chamber for the same time lost about 30 per cent. In man the glands on each side are rarely of equal bulk, but they contain loads of adrenalin which do not differ, as a rule, by more than 0·2 mg. from one another. The difference in weight of a pair of glands is generally caused by unequal development of the cortex; and a glance through the figures given later in this paper will recognize that glands differing from one another by 0·5 grm. in weight none the less contain, as a rule, almost identical loads of adrenalin. A striking example of this equality in difference is Case 79, 7·98 grm. = 1·4 mg. and 5·63 grm. = 1·25 mg. On the other hand, a few cases do exhibit marked inequality of the adrenalin content, as Case 65, 4·88 = 2·45 mg. and 3·41 = 1·9 mg.

This inconstancy is a serious obstacle to the attempt to measure the loss of adrenalin that occurs post-mortem in man. Six cases were analysed for this purpose, and by ill fortune they chanced to have glands of very unequal size, so that the conclusions to be drawn from the analyses are of the roughest nature.

Case 71.[7]	Gland 5 hrs.	post-mortem,	4·22 grm.	= 1.12 mg.	in 5 hrs.
	,, 10 ,,		4·38 ,,	= 1·0 ,,	10 % loss.
,, 19 a.	,, 4 ,,	post-mortem,	6·5 ,,	= 1·2 ,,	in 8 hrs.
	,, 12 ,,		6·44 ,,	= 1·1 ,,	8 % loss
,, 73.	,, 2 ,,	post-mortem,	4·27 ,,	= 4·0 ,,	in 2 hrs.
	,, 4 ,,		5·68 ,,	= 4·0 ,,	?
,, 82.	,, 4 ,,	post-mortem,	4·5 ,,	= 1·47 ,,	in 17 hrs.
	,, 21 ,,		5·92 ,,	= 2·8 ,,	?
,, 76.	,, 2 ,,	post-mortem,	3·1 ,,	= 3·2 ,,	in 18 hrs.
	,, 20 ,,		4·45 ,,	= 3·25 ,,	?
,, 72.	,, 2 ,,	post-mortem,	2·82 ,,	= 0·9 ,,	in 20 hrs.
	,, 22 ,,		6·65 ,,	= 1·25 ,,	?

Since in Cases 82 and 76 there was no demonstrable loss in eighteen hours, while the gland that was left in the body chanced to be the larger of the two and so may have contained originally more adrenalin, one may suppose that the loss in eighteen hours is not more than 10 to 15 per cent.

---

[7] Two of these cases were from cerebral hemiplegia. There was no evidence of a direct cerebral control of the adrenalin load of the gland on one side rather than on the other. Sir Victor Horsley and the writer made several experiments on cats in the hope of finding a cerebral centre or tract controlling the gland of one side only, but the investigation was fruitless.

Having thus obtained a rough indication of the loss of adrenalin that occurs in the glands post-mortem, one must next consider certain general factors that have been proved to cause rapid exhaustion of adrenalin in the few hours before death. These are much more important, insomuch as they are attended by greater exhaustion. Both the ante-mortem and the post-mortem conditions of loss in individual cases have been neglected by previous writers on this question, who have dealt rather with the relationship of the case to some particular malady. Death from disease in man is preceded by so much disturbance in mind and body that one cannot easily disentangle the separate causes in the summed effect. Consequently experiments were made to study each factor separately in animals, in which the control of the glands by the nervous system could at the same time be investigated. I found that in cats the exhaustion of adrenalin was almost entirely the result of excitant impulses passing down the splanchnic nerves, and that it could be prevented by section of these nerves. In my experiments the splanchnic nerves were cut on one side, and a week or more later the effect of the exhausting factor was determined by comparing the adrenalin loads in the resting gland on the side with cut nerves and in the other gland to which the exhausting impulses had access. Many and various were the conditions that led to loss of adrenalin, and even in a few hours this loss was very great. Emotional fright, as also shown by Cannon (10); simple anaesthesia of long duration, whether it was with ether or chloroform; excitation of afferent nerves, such as those of the great sciatic; poisoning with diphtheria toxin—all were effective, and in every case the action was through the splanchnic nerves, even in the case of diphtheria. So it was clear that the adrenal glands are played upon by the splanchnic nerves in the emotional and vasomotor reflexes with almost as frequent a call as are the muscles of the blood-vessels themselves. In such a crisis as impending death from diphtheria intoxication there is an urgent demand by the animal in its efforts to combat the fall of blood pressure and other changes due to action of the poison elsewhere. But even in diphtheria the adrenalin exhaustion is secondary, an expression of the cat's struggle for life: it is not a primary factor leading to death.

Since emotion, prolonged anaesthesia, cerebral haemorrhage with its sustained vaso-constrictor effort, and the like can rapidly exhaust the adrenalin from the gland in animals, it is evident that cases of death from disease in man, when changes in the glands are being studied, must be reviewed with close attention to the general conditions of life in the last few days, altogether apart from the special disease in question. The cases must be grouped in accordance with the main pathological disorders of the machinery of the body rather than under a catalogue of the special clinical diseases or the particular microbic infection that may be giving rise to these wider changes. In this respect the observations given in this paper have more value because, as a physician, I was personally familiar with the clinical history of the cases in which the glands were analysed, and myself saw the post-mortem examinations. Consequently I was able to form a fair opinion of the general pathological disorders arising in the progress of each disease. In only a few examples of children with fevers were glands analysed from cases in which I had not seen the autopsies. This was material which was not readily obtained at University College Hospital, and for it I am indebted to the kindness of Dr. E. W. Goodall and of Dr. A. C. B. Biggs.

The adrenalin content was measured in eighty cases; and the cortical lipoid was examined by the microscope in 170 cases. These were selected to the extent that straightforward simple cases were chosen as far as possible and complications avoided. The weights of the glands and the adrenalin load are always given separately for each gland of a pair. Most writers add the two glands together. The medulla is aggregated chiefly in a mass at the venous pole of the gland, and it rarely extends between the cortical laminae at the other pole. Adrenalin is not yielded by extraction of this cortical end, and therefore in a few cases, where fat content was required as well as adrenalin load, a small piece was cut out of the gland at this end and microscoped.

The results are described more or less separately for the cortical lipoid and the adrenalin. As yet there is no physiological proof that these two substances are related to one another, and their pathological changes do not exhibit any close parallelism. The cases quoted in the next pages are classified in the first place with regard to their load of cortical lipoid. To save repetition of clinical detail, the adrenalin content is given at the same time where this had been determined. But the conditions of adrenalin exhaustion are summarized in a later part of the paper.

Normal Glands.

The standard of a normal gland can only be determined by examining the glands taken soon after death from a healthy man who has died suddenly and unexpectedly, and who was subjected neither to emotional alarm nor to anaesthesia. Such material is not easy to procure on account of the delay generally caused by the necessary inquest. I examined five glands from two to seventeen hours after death. All showed a superabundant load of lipoid in the cortex, the substance being so rich that it did not stain deeply with Scharlach but gave a glittering field of light through the crossed polarizing prisms. Two of these were taken from healthy men killed in railway accidents.

Case 1. Man, aged 44. Cervical spine crushed by railway engine as he was going to work in the morning after a full night's rest: death was instantaneous.

Adrenal glands analysed six hours post-mortem,

4·1 grm. containing 4·0 mg. adrenalin.
3·85 grm. containing 4·1 mg. adrenalin.

Two others were from ruptured aneurisms, in cases which were in good general health and had no septic fever; and the fifth was from a man with a small tuberculous lesion in the lung, who had been progressing most favourably without fever or loss of weight, but who died in a few minutes from a profuse haemoptysis.

Case 2. Man, aged 48. Aneurism pressing on bronchus and oesophagus; no fever and no pain. Rupture into oesophagus and speedy death.

Glands 17 hrs. post-mortem, 3·72 grm. = 5.0 mg. adrenalin.
3·74 grm. for microscope.

Each normal gland of an adult in fair health should weigh about 4 grm., and contain from 4 to 5 mg. adrenalin:[8] the cortex should be loaded with doubly refractive lipoid throughout. Schmorl and Ingier found an average yield of only 2·35 mg. for each gland in fifteen normal cases, which illustrates the insufficiency of their method of analysis.

Starvation.

Slow inanition leads to emaciation and complete loss of body fat. The cortical lipoid is in nowise reduced in this process. Its storage and its loss are determined by conditions altogether different from those controlling the general fat dépôts of the body. As will be seen later, septic infections cause it quickly to vanish, whereas simple conditions of bodily emaciation leave it totally unaffected.

Case 3. Girl, aged 17. Died in six months of anorexia nervosa, she having apparently assumed this hysterical state by imitation of a sister. No fever. Body wasted almost to a fleshless skeleton, weight 3 st. 6 lb.; no lesions found post-mortem. Adrenals crammed with lipoid to extreme degree; adrenalin not measured.

Case 4. Woman, aged 29. Died in eight months of anorexia nervosa. Body so wasted that no fat was seen anywhere post-mortem; ordinary height, and weight 3 st. 10 lb. In the last week there was slight fever from gangrenous broncho-pneumonia, and this must have tended to reduce the lipoid and the adrenalin, and to increase the weight of the glands, which were otherwise normal.

Glands 14 hrs. post-mortem, 5·82 grm. = 2·0 mg.
5·2 grm. = 2·0 mg.

Fat plentiful, but already escaping from the cells by early autolysis. The fat in the corpora lutea was full and normal.

Case 5. Man, aged 39. Dysentery and ulcerative colitis for years; very emaciated and feeble. Died of incessant diarrhoea, without fever. All organs shrunken, and heart weighed only 6 oz. (170 grm.).

Glands 10 hrs. post-mortem, full of doubly refractive fat.
4·8 grm. = 1·8 mg. adrenalin.

Case 6. Child, aged 8 weeks. Death by inanition from hypertrophic pyloric stenosis. Glands filled with doubly refractive fat.

---

[8] This 1 : 1,000 proportion of adrenalin to the weight of the entire gland, cortex as well as medulla, oddly holds good also for the cat—average for each gland 0·2 grm. with 0·22 mg. adrenalin—and even for cattle. Parke Davis & Co. informed me that 1 kilogram of slaughter-house glands are needed to yield 1 grm. of pure adrenalin.

Similarly in thin diabetics and in cancer of the stomach, where the emaciation is extreme, the load of cortical lipoid is none the less full.

In this reference may be quoted Federici's (21) observations that there is no change in the cortical fat of bats or dormice relative to the state of hibernation; and further, that starvation to death does not alter the fat of a guinea-pig's gland.

Simple Heart Failure.

Broadly, death by heart failure, when it is not caused by disease elsewhere in the body, may occur in one of three forms:

(*a*) Sudden stoppage of the heart, that is presumably by ventricular fibrillation. Cases of pulmonary embolism or auricular thrombosis are not essentially faults of the heart.

(*b*) Rapid dilatation and failure of the heart, associated with a pulsating liver and some cyanosis, but often only slight dropsy. The patient dies in a few days, and may suffer much mental distress in the acuteness of the collapse.

(*c*) Progressive failure of the circulation with general water-logging and an apathetic death by slow degrees.

Three cases of (*a*) were examined from patients with mitral stenosis and auricular fibrillation, and one from aortic regurgitation. The glands had their normal load of lipoid and of adrenalin. On account of the emotional distress and the effort made by the patient to restore the fast-failing circulation, (*b*) might be expected to reveal some demand on the adrenal glands. Only two examples were analysed and in them the lipoid was almost normal, but the adrenalin was much reduced.

Case 7. Girl, aged 16. Aortic and mitral disease of old rheumatic origin. Attack of purpura three weeks before death, followed by fatty degeneration of the heart and complete breakdown in last week: final acute collapse with great distress in last thirty hours, subnormal temperature and imperceptible pulse.

Glands 9 hrs. post-mortem, fairly loaded with lipoid.
4·35 grm. = 0·7 mg. adrenalin.

Case 8. Woman, aged 26. Ill for three years with mitral regurgitation from an hypertrophied feeble heart (23 oz. = 650 grm.). Blood pressure never raised. Complete failure in last few days with oedema, cyanosis, cold sweat, feeble though regular pulse (120) and much mental distress. No valvular lesion found. No atheroma: kidney normal.

Gland 8 hrs. post-mortem, 4·7 grm. = 0·2 mg.
Other gland of same size and full of lipoid.

(*c*) gave variable results in the five cases examined, but the lipoid was often in part exhausted, and there was generally a round-celled infiltration of the gland.

Case 9. Man, aged 35. Mitral stenosis and auricular fibrillation. Very slowly his circulation became impeded by thrombosis within the dilated auricles. The left auricle was distended with clot, which spread into the lungs causing extensive infarction for at least a week before death. No mental distress. The patient was semi-comatose, with a very cold skin, for the last two days. Kidneys only congested. Aorta small and free from atheroma, but pulmonary arteries very atheromatous from old-standing obstruction.

Glands 2 hrs. post-mortem, fat normal. Weight increased by slightly haemorrhagic congestion.

5·42 = 2·3 mg.
5·78 = 2·3 mg.

More cases need analysis under this last grouping, but the main result is clear, that simple cardiac failure is not associated with loss of lipoid, though it may be with exhaustion of adrenalin, where the patient has been fighting hard for life.

Pneumonia.

Of this disease eight adult cases were examined. Three were acute, with death in three days; and they showed the most marked changes of all the glands that were ever examined. They were utterly exhausted of lipoid, and much enlarged by general oedema.

Case 10. Man, aged 37. Pneumonia for three days. Temperature 101°. Lungs, soft purple consolidation.

Glands 5 hrs. post-mortem, 11·6 grm.
10·35 grm. = 1·45 mg. adrenalin.

Lipoid completely gone, and the cortical cells looked very small, as though they were compressed by the general oedema of the tissues. The glands were twice their normal size.

In more prolonged cases of pneumonia the oedematous enlargement and the exhaustion of lipoid were less, even though death came with cyanosis and the rapid pulse of heart failure. For example, three patients who survived to the seventh or tenth day had glands which weighed 6 and 7 grm. each, and contained a little fat.

Case 11. Man, aged 42. Pneumonia of right side, seven days. Toxic collapse with strain of being brought to hospital.

Glands 22 hrs. post-mortem, fat almost gone.
7·4 grm. = 1·2 mg. adrenalin.

Case 12. Man, aged 39. Right lung, eight days. Temperature 104°. Grey cyanosis.

Glands 10 hrs. post-mortem, 6·0 grm., very little fat.
6·7 grm.

Case 13. Man, aged 42. Pneumonia complicating syphilitic aortic regurgitation. Ill six days. Heart 23 oz. Evidence in liver of slight chronic heart failure. Pneumonia both bases.

Glands 4 hrs. post-mortem, swollen, and only a little fat in inner zone.

7·23 grm. = 2·5 mg.
6·72 grm. = 2·5 mg.

But in two cases, aged 50 and 67, of the so-called 'asthenic' type, which lived twelve days and showed practically no rise of temperature at all, though the lungs were consolidated with a puriform exudate, the glands were of normal size and contained a fair amount of fat.

Similarly in a woman of 36, who shortly after induction for placenta praevia died with bronchitis and cyanosis that developed to the fatal issue in four days but was not accompanied by a temperature above 99°, while there was neither pneumonia nor uterine sepsis, the glands had a tolerable load of lipoid. In this last example, such loss of fat as had occurred in the last two or three days of life was prettily shown, because venous thrombosis had occurred before death at one pole of the gland and in this area, in which degenerative changes had not had time to take place, the accumulated lipoid was richer and more anisotropic than in the rest of the gland.

Of children, aged from 7 months to 2 years, five examples were studied, with pneumococcal infection of lungs, pleura, meninges, or peritoneum. All showed considerable exhaustion, fat being left only in the thin outer rim of the gland.

Acute Fevers.

Measles (two cases) and scarlet fever (three cases) showed rapid exhaustion of fat in the acutely fatal cases, just as in pneumonia: scarlet fever is distinguished by a special round-celled infiltration through the gland. The glands are exhausted in young children much as in adults, but in them there is a marked tendency for the fat to cling longest to the outer rim, the zona glomerulosa; that is, to the outer rim of the true cortex, not to the entire ring of true cortex enclosing the degenerating foetal tissue (19), for this latter has all disappeared at the age of one year, and is indeed very small at the age of six months. E. Thomas (43) stated that there was no loss of cortical fat in children with measles or scarlet fever, and in that he was certainly wrong. Weltmann (47) thought that the exhaustion, though quite evident, was less readily produced in children than in adults. I have seen no reason to assent to this opinion, except in regard to the thin outer fat-holding rim.

In very young children it is interesting to compare the behaviour of the true cortical lipoid with that of the degenerating fat. The latter is present in the dying cells of the central remnant of the foetal cortex. With bronchopneumonia, gastroenteritis, or any other such febrile cause of death, the cells of the true cortex are exhausted of lipoid: but the degenerative fat of the inner area shows no change, although there is full circulation in the capillaries around it.

Diphtheria does not produce the same change as scarlet fever or measles. It is a disease of great toxicity, but little pyrexia; and it is not attended by marked loss of lipoid. Three cases were those of death associated, so to speak, with the accidents of diphtheria. The first (1½ years old) was a laryngeal diphtheria with death on the second day by haemorrhage during an attempted tracheotomy: the glands were absolutely normal and loaded with doubly refractive lipoid. The second, in a child of 5, was again laryngeal, with death on the third day by simple asphyxia: once more the lipoid was almost normal. Lastly was a case of post-diphtheritic neuritis.

Case 14. Child aged 2 years and 8 months. Faucial and laryngeal diphtheria. Good recovery, and then death on thirty-fifth day with asphyxia and early broncho-pneumonia after two days' paralysis of diaphragm.

Glands 4 hrs. post-mortem, fat full.

1·56 grm. = 0·7 mg. adrenalin.

Thus in these simple asphyxial deaths the lipoid was present in abundance, and in the first two it was unaffected by the slight diphtheria poisoning of the body.

In contrast with them were three cases of the extremely toxic type of haemorrhagic diphtheria, aged from 3½ to 6 years, and dying from the fourth to the eighth day. Here the cortical lipoid was moderately exhausted, very much less so than in scarlet fever or pneumonia. Still, there was some exhaustion, so that the cortical cells were taking only a blue stain at the very time when the kidneys and heart were stained red with fatty degeneration.

Case 15. Child, aged 3½ years. Haemorrhagic naso-pharyngeal diphtheria. Ill two days. Pulseless when admitted to hospital and died one hour later. Superficial and deep haemorrhages.

Glands 17 hrs. post-mortem, fat fair.

1·4 grm. = 0·37 mg. adrenalin.

To complete the account of the children, details may be given here of the conditions of marasmus to which reference has already been made. In four cases of gastro-enteritis, which lived from five to twenty-one days and all displayed fever until the final collapse, the fat was lost from the true cortex.

Case 16. Child, aged 2 years and 8 months. Diarrhoea for one week following bronchitis. Temperature 101°.

Glands 12 hrs. post-mortem, 1·71 grm. fat lost.

1·62 grm. = 0·31 mg.

Case 17. Child, aged 1 year and 2 months. Diarrhoea and vomiting for three weeks and slight bronchitis.

Glands 5 hrs. post-mortem, a little fat only in outer rim.

On the other hand, in three children which died in the first year from wasting without fever or any ascertainable cause, and in one case of hypertrophic pyloric stenosis (6), the cortex contained a considerable amount of lipoid.

Various Septic Conditions.

Seven cases were examined, and all showed much exhaustion of lipoid, whether the disease were brief or of long duration. Thus an influenzal septicaemia with fever of 98° to 102° for two months in an old man of 74, and a staphylococcal pyaemia of five months, had both utterly fatless glands.

Case 18. Man, aged 29. Staphylococcal pyaemia for five months, and perinephritic abscesses developing from foci in kidney. Continuous fever and very emaciated.

Glands 20 hrs. post-mortem, no fat, but rather oedematous.

7·72 grm. = 2·5 mg. adrenalin.

A chronic meningitis of five weeks, and another of three days, both with fever, were equally exhausted of all lipoid.

Case 19. Boy, aged 13. Chronic septic meningitis from middle ear. Fever to 103°.

Glands 5 hrs. post-mortem, fat exhausted.
4·75 grm. = 2·0 mg. adrenalin.

But two milder cases, a chronic empyema and an actinomycosis of the chest, showed incomplete exhaustion.

Case 19a. Fully grown girl, aged 16. Suppurative meningitis from otorrhoea, five to six days: fever 103°. Glands oedematous, but not haemorrhagic; fat largely exhausted.

Right, 4 hrs. post-mortem, 6·5 grm. = 1·2 mg. adrenalin.
Left, 12 hrs. post-mortem, 6·44 grm. = 1·2 mg.

Malignant Endocarditis.

This disease combines heart failure with toxic septicaemia and prolonged fever: the first factor has been shown to have only slight influence, if any, on the cortical lipoid, whereas septic fever in general causes exhaustion. Six cases were examined, and the lipoid exhaustion was well marked in all, though never so complete as in pneumonia, nor were the glands oedematous and enlarged as in the latter disease. Two of the cases had repeated rigors, dying after illnesses of only eight and twelve weeks and presenting the clinical appearance of profound sepsis; yet there was a little lipoid to be found in each under the microscope. A rapid streptoccocal case, with death in five weeks, was the most exhausted of all.

Case 20. Woman, aged 37. Ill twelve weeks with very septic infection of aortic valves. Temperature 103°, and rigors.

Glands 13 hrs. post-mortem, fat largely exhausted.
6·47 grm. = 2·1 mg. adrenalin.

Case 21. Woman, aged 57. Mild septicaemia for ten weeks with fever 99 to 100°.

Glands 8 hrs. post-mortem, fat moderately exhausted.
3·72 grm. = 2·3 mg. adrenalin.

Case 22. Woman, aged 22. Mild endocarditis for five months, and uraemic death by toxic nephritis and dropsy with fever.

Glands 20 hrs. post-mortem, fat fair; a little oedematous.
6·3 grm. = 0·75 mg. adrenalin.
4·7 grm. = 0·35 mg. ,,

Case 23. Woman, aged 23. Ill six months with continual fever. Temperature 102°.

Glands 8 hrs. post-mortem, 5·39 grm. = 0·75 mg.
5·37 grm., fat only in small areas.

Though rarely complete, the loss of yellow fat from the cortex was always so manifest to the naked eye that this change in the adrenals at an autopsy is as valuable a suggestion of the septic nature of a doubtful case of endocarditis as is an enlarged spleen, for the glands from death in ordinary heart failure carry an abundant load of lipoid.

Nervous Diseases.

In the simple cases of death from damage of vital nervous centres, whether it be the heart or the respiration that fails, there is naturally no exhaustion of the lipoid. Thus a girl of 18 died with an afebrile ascending myelitis of the spinal cord, respiration being paralysed on the ninth day: the glands showed normal fat and a full chromaffine yellow.

Case 24. Man, aged 52. Subacute combined degeneration of the spinal cord for ten months. Rapid collapse and death by heart failure in twenty-four hours, presumably by the development of a bulbar focus of myelitis. No fever.

Glands 5 hrs. post-mortem, 5·57 grm., with fair load of lipoid.
4·9 grm.

Case 25. Man, aged 55. Pachymeningitis haemorrhagica on left side, with headache for ten and coma for two days. Slight fever on last day. Kidneys normal.

Glands 6 hrs. post-mortem, 4·95 grm. = 3 mg.
4·85 grm., and very full of fat, giving on chemical analysis the highest percentage of all examined.

Carcinoma.

Death comes in so many ways to the victims of cancer, that it serves but little purpose to collect many examples under this heading. In simple cases, where there was no outspoken secondary sepsis and the patient succumbed rather to inanition, the glands were always well loaded with lipoid and with chromaffine substance. Thus in three subjects of cancer of the stomach, with death by vomiting and extreme emaciation, the adrenals were full of fat. Secondary deposits were very frequent in the glands, occurring in the first place in the medulla. As these spread outward into the cortex, their compression of the nearest cortical cell columns checked mechanically the formation and storage of fat by the latter, which therefore took only a blue stain close to the growth. In several sections the growth in the cortex seemed to be lying within the heart of the cortical cell columns, distending the potential lumen that was described earlier as being engorged with blood in glands where the central vein was thrombosed.

Case 26. Woman, aged 42. Cancer of lungs with outgrowths causing pleural and pericardial effusions. Ill four months. Sudden death by pulmonary embolism, during quiescent period after slight fever.

Glands 18 hrs. post-mortem, full of lipoid.
6·32 grm. = 4·2 mg.

This was practically a case of sudden death in a cancerous patient, and it illustrates well the fact that the glands are not affected by the simple growth of cancer in the body.

The next case, in which a man died in slow asphyxia with great cyanosis and dyspnoea but no fever, proves, together with Case 14 of diphtheritic laryngitis, that asphyxia does not affect the lipoid of the cortex.

Case 27. Man, aged 44. Sarcoma of thyroid with very large and numerous secondary deposits in the lungs, causing a slow asphyxial death. No fever.

Glands 11 hrs. post-mortem, well filled with doubly refractive lipoid.

Tuberculosis.

In view of what has been recorded for other fatal diseases it would be natural to expect that acute tuberculosis with high fever, or tuberculosis complicated by secondary septic infections, would be especially associated with loss of cortical lipoid; and that the more chronic tuberculous changes, however great the bodily emaciation produced, would lead to less exhaustion. Such was found to be the case in the nineteen examples of this infection.

Case 28. Man, aged 45. Pulmonary tuberculosis eighteen months, with rapid development at end and persistent fever, 99° to 102°.

Glands 11 hrs. post-mortem, normal size. Fat quite exhausted.

Case 29. Woman, aged 36. Old tubercle at apex, which spread and caused rapid death by miliary tuberculosis of both lungs eight days after confinement at sixth month. No fat in lutein cells of ovary.

Glands 22 hrs. post-mortem, 5·4 grm., very little fat.
5·81 grm.

Case 30. Boy, aged 12. Miliary tuberculosis of lungs, liver, spleen, kidneys, and brain. Persistent fever, so that it was mistaken for some time as typhoid.

Glands, a very little fat in outer rim.

Case 31. Child, aged 1 year. Miliary tuberculosis and meningitis: two weeks' fever to 100°.

Glands 5 hrs. post-mortem, a little fat only in outer rim.

Case 32. Woman, aged 45. Tuberculosis of lungs and intestines: three weeks' fever 100° to 102°, and terminally enteritis and laryngeal dyspnoea.

Glands 8 hrs. post-mortem, oedematous and a little scattered fat.
8·8 grm. = 2·14 mg.

Case 33. Man, aged 28. Miliary tuberculosis of lungs and meninges, resembling typhoid. Temperature 100°. Death in three weeks.

Glands 24 hrs. post-mortem, 7·05 grm., fat fair.
7·8 grm. = 1·4 mg.

The damage of the glands in Addison's disease is a familiar statement in pathology, both cortex and medulla being destroyed by the fibro-caseous inflammation. So little cortex is left at the time of death, that one might fairly argue that the cortex as well as the medulla must be destroyed before the illness puts an end to life. The process is chronic and afebrile; and in the few cortical cells that may chance to escape the general inflammation fat can still be detected, just as there may be still a trace of adrenalin in the gland. But in some rapid cases, where the adrenals are infected and destroyed in the course of a generalized active tuberculosis, there is fever and the few surviving cortical cells are empty of fat.

Case 34. Man, aged 25. Addison's disease for six months. No fever. Slow death by general weakness. No active tubercle elsewhere.

Glands 6 hrs. post-mortem, enlarged and fibro-caseous; no medulla left; the few cortical cells that had escaped still stained brilliantly with Scharlach (Fig. 4).

Case 35. Man, aged 29. Old, healed tuberculous caries of spine. Addison's disease for rather less than a year. No fever, and no tubercle elsewhere.

Glands 7 hrs. post-mortem, enlarged, caseous and cretaceous.

10·1 grm. = 0·03 mg.

Case 36. Man, aged 34. Chronic Addison's disease for ten years. Developed pulmonary phthisis and died in two months, the temperature being always subnormal despite the disease in the lungs. Adrenals of extremely atrophic type, so that they could not be dissected out and weighed. No accessories discovered.

Glands 6 hrs. post-mortem, one side assayed for adrenalin = 0. Other side: a few giant cell systems and areas of inflammation. No medulla seen. Ganglion cells normal. Few clumps of cells, containing brown pigment but no fat, being apparently cortical.

Case 37. Man, aged 51. General tuberculosis for some months. Fever 99° to 100°. Weakness and pigmentation in last few weeks. Old tuberculosis of mesenteric glands; miliary tuberculosis of lungs, liver, and kidneys.

Glands 4 hrs. post-mortem, shape preserved, but only a few nodules of live cortical cells seen at the periphery, and from these the lipoid was gone. Much of the cortex showed its normal columnar structure and was free from tuberculous inflammation; but its cells were necrosed, either from recent tuberculous poisoning or from vascular obstruction.

Anaemia.

Up to this point all the fatal maladies, which have been discussed in this paper and shown to be associated with loss of the cortical lipoid, have had one feature common to them, namely, rise of temperature. This section introduces a fresh group. Eight cases were examined of death by haemorrhage from gastric ulcers, either acute or chronic. In all of these death was the result of repeated haemorrhage extending over several days, and in the last two or three days there was generally mild delirium with slight fever. The heart and the liver often showed degenerative increase of fat under the microscope, but the lipoid load was definitely reduced in all the adrenals, though never to complete exhaustion. This was the result, whether the temperature had been febrile or normal. The glands were a little heavier than normal, averaging 6 grm. each.

Case 38. Man, aged 30. Chronic duodenal ulcer with symptoms for eighteen months. Death by haemorrhage recurrent for twenty days, with mild delirium in last three days. Temperature 98° to 99°. Pulse 120. Saline infusions were used freely, so that at death the body contained a fair amount of blood. Kidneys normal, and their veins not thrombosed.

Glands 1½ hrs. post-mortem, fat exhausted, and recent haemorrhage on both sides at cortico-medullary zone, so that the glands were much enlarged. Neither cortex nor medulla was actually ploughed up, but the effused blood lay in a large clot between them. Fair chromaffine yellow.

Case 39. Man, aged 50. Chronic gastric ulcer with pain for five months and haemorrhage; very anaemic. Heart showed 'tabby cat' striae of fatty degeneration.

Glands, fat largely exhausted, but chromaffine a good yellow.

Case 40. Woman, aged 69. Recurrent haemorrhage for two weeks from two acute gastric ulcers. No fever. Delirium in last two days. Fibrosed kidneys.

Glands, fat practically exhausted: large cortical adenomata.

The next instance (Case 41) was complicated by a mild septic endocarditis, which may by its toxic influence have caused the fresh bleeding from the chronic ulcer: death seemed to be due to the haemorrhage rather than to the septicaemia.

Case 41. Woman, aged 33. Chronic gastric ulcer with haemorrhage for ten days, and mild delirium for three days; fever of 102°. Recent tricuspid endocarditis and septic spleen.

Glands 7 hrs. post-mortem, 5·05 grm., very little fat left.
6·7 grm. = 3·25 mg.

Two other cases, in contrast with those already quoted, showed a moderate load of lipoid and a fair chromaffine yellow. One was from an acute ulcer, where a slight bleeding was eight days later succeeded by a big haemorrhage, that continued and was fatal in a few hours; and the second was a chronic gastric ulcer that bled repeatedly for sixteen days while the patient, a man of 51, was heavily drugged with morphia and atropin (T. 99° to 100°. Gland 6·45 grm., with a fair load of fat).

Similar to these two was a clear case of splenic anaemia with death from repeated haemorrhages and terminal thromboses in the portal veins; and in this, too, there was no loss of fat.

Case 42. Man, aged 30. Repeated haematemesis and melaena for last eight months of life. Spleen much enlarged. Liver unchanged. No ascites. Blood extremely watery. Mild delirium in last three days. Thrombosis of superior mesenteric veins.

Glands 8 hrs. post-mortem, abundant lipoid.
4·26 grm. = 2·5 mg.

The group of anaemias from haemorrhage is, therefore, one which behaves inconstantly. In many cases the fat is exhausted; in others it shows no change. The factor which determined this difference could not be recognized.

Leukaemia.

The condition in this malady is complex. There is the tumour-like overgrowth of the leucocytes, whether floating or massed in various organs: there is often fever and secondary anaemia. The last two factors in themselves have a definite exhausting influence on the cortical lipoid; though it must be noted that the fevers, which so far have been shown to be associated with exhaustion, were of external septic origin, and not caused by intrinsic changes in the body itself, as seems to be the case with the febrile phases of leukaemia.

Only five examples were analysed. Four were acute cases, accompanied by fever and resulting in death within six months. Of these one was a child of 4½ years, which practically bled to death from purpura of the alimentary canal; the other three were adults, two lymphatic and one spleno-medullary. In all, the adrenals were largely exhausted of lipoid, though, as in the secondary anaemias, fat might appear in excess in the liver and heart. The adrenal glands were in no case enlarged, for, though lymphocytes were present in their capillaries, they did not lodge within the gland and form germinating areas as in the kidney or liver.

On the other hand, a very chronic case of lymphatic leukaemia in an old man of 69, with a high leucocyte count of 500,000 cells to the c.mm., showed very slight loss of lipoid. This example, however, was complicated by an opposing factor, fibrosed kidneys with high blood pressure, so that it is hardly worth quoting in this respect.

Post-operative Shock and Death.

It serves no purpose to summarize the different views that are held with regard to the nature of 'shock' as the cause of death following an extensive operation. My observations on cats proved that a long anaesthesia, or stimulation of afferent nerves, can each exhaust adrenalin from the gland, but the lipoid was not found to be much changed under such conditions. In five cases in man where a prolonged operation was followed by shock and death within twelve hours, the lipoid was present in abundance, and there was only moderate reduction in the load of adrenalin.

Case 43. Man, aged 57. Tongue excised for cancer. One week afterwards the glands of the neck were removed in an extensive dissection of three hours under intravenous ether. The patient died 1½ hours later of heart failure.

Glands 5 hrs. post-mortem, most abundant lipoid. 3·59 grm. = 3·6 mg.
3·7 grm. = 3·6 mg.

Case 44. Woman, aged 47. Wertheim's operation for cancer of uterus. Thirteen days later the vagina was removed under intravenous ether. The operation lasted forty-five minutes, but the patient never rallied and died eight hours afterwards. No fever.

Glands 8 hrs. post-mortem, 4·4 grm., fat most abundant.
4·76 grm. = 3·2 mg.

Case 45. Woman, aged 38. Optic neuritis, which was probably due to renal disease without high blood pressure or other manifest features, led to mistaken diagnosis of cerebellar tumour. Occipital decompression under open ether, 1½ hrs. Dura not opened, but patient lost much blood and became pulseless during the operation: she remained comatose and died fifteen hours later with a final temperature of 107°.

Glands 12 hrs. post-mortem fat abundant.
3·63 grm. = 1·5 mg.

Case 46. Girl, aged 13, in good health. Plastic operation for two hours under chloroform to restore contour of face, which had been disfigured by scars of old cancrum oris. Death as soon as patient left the operating theatre, from a blood clot in the trachea. Before the operation temperature, pulse, and respiration were normal, so that the patient was probably free from anxiety.

Glands 18 hrs. post-mortem. fat irregular.
2·65 grm. = 1·8 mg.

These examples prove that an anaesthetic leaves the lipoid almost unchanged, and Case 45 shows further the important point that a rapid non-septic rise of temperature to 107° also does not cause it to vanish.

Ductless Gland Disease.

These diseases are thrown together in reference to the prevailing theory that the ductless glands form a mutually dependent ring, in which damage of one is bound to affect the activities of the rest and lead to the phenomena of ‘pluriglandular insufficiency’.

Diabetes, with death by coma, supplied five cases. Of these three were extremely emaciated, while two were better nourished, but succumbed to secondary infections in the lungs. The adrenals were simply normal in size and they contained a fair load of lipoid except in the two cases of secondary infection, so that the loss in the latter was evidently related to the septic state and not to the diabetes or emaciation.

No tendency to an increase of the cortical lipoid, like that in kidney disease, was observed; though Klinkert (28), using Windaus's digitonin method of analysis, found a definite increase of the cholesterin in the blood in cases of diabetes mellitus with acidosis (0·38 per cent. instead of the normal 0·17 per cent.).

Case 47. Woman, aged 35. Diabetes for one year and coma for thirty-six hours. Not emaciated.

Glands 20 hrs. post-mortem, fat fair load.
4·53 grm., medulla very small.

Case 48. Woman, aged 25. Diabetes about six months, bronchitis and tuberculosis. Temperature only 97°. Very wasted. Pancreas not fibrosed, and cause of diabetes doubtful.

Glands 23 hrs. post-mortem, fat moderately abundant, but not in anisotropic form.

Case 49. Man, aged 28. Diabetes one year, tuberculosis and pyoneumothorax for four months, but only slight pyrexia. Very thin. Pancreas fibrosed.
Glands 18 hrs. post-mortem, fat, moderately exhausted.

Case 50. Man, aged 62. Diabetes and gangrene of foot with coma. Body well nourished and reeking of acetone.

Glands 8 hrs. post-mortem, yet remarkably autolysed.
Fat very abundant. 6·8 grm. = 2 mg.

Pituitary disease furnished only one example, and this did show pluriglandular changes. Acromegaly had developed ten years earlier. Ultimately some features of subpituitarism appeared as the gland enlarged into a sarcoma-like growth that compressed the frontal lobes. The thyroid grew bigger and cystic degeneration within its substance became so extensive as to cause difficulty of breathing. The adrenals were enlarged.

Case 51. Man, aged 41. Acromegaly for ten years, and latterly patient became blind in one eye and rather obese, while the symptoms of an intracranial tumour declared themselves. The thyroid was so enlarged by colloid cysts in the last two years, that hemithyroidectomy was first performed for the dyspnoea. Later an attempt was made to explore the pituitary fossa by intranasal operation. This took three hours under chloroform and did not cause great shock, but the patient died suddenly an hour later by asphyxia from blood in the windpipe.

Glands 15 hrs. post-mortem, 7·83 grm. = 4·2 mg.
6·0 grm. + a lost fragment; moderate load of lipoid.

Perhaps there was not enough evidence for regarding the ultimate condition of the man as that of subpituitarism, for his obesity might have been simply the result of the sedentary life[9] imposed by his blindness. But the pituitary[10] was at any rate changed, and with this was undeniable overgrowth of the thyroid and of the adrenals. The latter seemed to be symmetrically enlarged, but the adrenalin load was not much increased, if one may argue from Cases 43, 44, and 46 that the loss under the anaesthetic and the operation had probably not exceeded 1 mg.

Reinhardt and Creutzfeldt (39) mention hypertrophy of the adrenals in a case of acromegaly, but they do not support the statement by giving the weight of the glands.

In *thyroid* disease the evidence was uncertain, though it suggested enlargement of the adrenals. A child that was born two weeks prematurely from a mother with pronounced exophthalmic goitre and itself displayed the disease (48) died on the second day from cerebral haemorrhage caused in delivery. Its glands were normal, 2·42 and 2·23 grm., but the paraganglion aorticum was a little enlarged. A woman of 23, with exophthalmic goitre, who died during an operation for removal of part of the gland, had adrenals with a normal amount of fat and slightly increased weight, 5·2 and 6·1 grm. And a third example was in a woman of 29, with acute enlargement of the thyroid in the last three months of pregnancy, but no obvious phenomena of exophthalmic goitre. She died three days after confinement in a state of asphyxia; and while one gland weighed 5·5 grm., the other was increased by a large central adenoma of the cortex to 9·3 grm. Such adenomata may, however, occur in any condition of health or disease. The lipoid in both was practically normal. In none of these was the adrenalin load measured.

Case 51 a. Woman, aged 28. Exophthalmic goitre with dyspnoea and tachycardia for 4 months. Vomiting developed and was fatal in 4 days; pulse 180; no fever. Pituitary, 0·53 grm., and containing no colloid.

Glands slightly oedematous; no adenomata; fat almost exhausted.
24 hrs. post-mortem, 6 grm. = 2·5 mg. adrenalin.
5·75 grm. = 2·5. mg.

---

[9] I have seen a man whose weight increased from 13 to 17 stones in eighteen months after the excision of one adrenal gland, but the corpulence in this case was almost certainly caused by his having taken up an indolent occupation.

[10] A 20 per cent. extract of the tumour contained no pressor substance of the type of pituitary extract.

Finally, one may mention at the end of this section an irrelevant case of pure toxaemia of pregnancy.

Case 52. Woman, aged 30. Confinement at full time; albuminuria, jaundice, and coma, without fits, at once set in, and culminated in death 48 hours after the birth of the child. Liver necrosed.

Glands normal size; no thrombosis or necrosis; outer half fully loaded with lipoid.

Kidney Disease.

The relationship of kidney disease to changes in the adrenal glands, both in the cortex and medulla, has been the subject of much theoretical discussion. This will be considered later on (pp. 82 to 87), and here only the simple facts are narrated as regards the cortical lipoid.

Patients with renal disease, in addition to dropsy, high blood pressure, and uraemic intoxication, may suffer with secondary infections. The latter condition in itself is likely to exhaust the lipoid, and therefore examples must be taken first where death is the direct result of the kidney mischief.

A. *Contracted kidneys with high blood pressure.*

Case 53. Man, aged 51. Small, fibrosed red kidney from lead poisoning ten years ago. Ill with headaches for two years. The case was watched for the last year, when the blood pressure was 230 to 200 mm., and it slowly terminated in a pure uraemic death with wasting, coma, and a little vomiting in the last seven days. Athero-sclerosis well marked in aorta. Heart 17 oz.

Glands 8 hrs. post-mortem, 4·5 and 4·2 grm. respectively.

No cortical adenomata. Lipoid extremely abundant. Medulla certainly not enlarged.

Case 54. Man, aged 68. Ill for one year. Blood pressure 170–80 mm. Sudden death in twenty-four hours by thrombosis of basilar artery. Temperature rose from 97° to 102°. Fibrosed, small kidneys. Heart not hypertrophied, and very little atheroma.

Glands 18 hrs. post-mortem, 3·21 and 3·2 grm.

Fat abundant, no cortical adenomata; medulla rather large.

Case 55. Woman, 61. Generalized arterio-sclerosis, the kidneys being moderately diseased. Blood pressure 200; heart 22 oz.; aorta very atheromatous. Kidneys 4 oz. each. Death by cerebral degeneration and coma, rather than by uraemia. Liver shrunken to 31 oz., yet adrenals enlarged.

Glands 7 hrs. post-mortem, 7·22 grm. = 4·3 mg.
6·74 grm. = 4·3 mg.

Fat very full, and many small adenomata.

Case 56. Man, aged 38. Lead poisoning twenty years ago. Two years of chronic uraemia with vomiting. Blood pressure 270 mm. Death with coma and blindness developing progressively in last three weeks. Temperature subnormal. Small red kidneys, 2 oz. each. Heart 13½ oz. Slight atheroma of aorta.

Glands 12 hrs. post-mortem, lipoid very abundant.

Small cortical adenomata. Medulla unusually small. 5·64 grm. and 5·32 grm.

Case 57. Woman, aged 50. Ill for two years with chronic uraemia, and acute development with fits, drowsiness and scanty urine for last nine days.

Blood pressure 170 mm. Temperature subnormal. Small white kidneys 2½ oz. each. Heart 13 oz. No atheroma. Slight pleurisy and pneumonia.

Glands 6 hrs. post-mortem, lipoid abundant and small adenomata; medulla extremely small.

5·56 grm. = 0·4 mg.
6·89 grm. = 0·4 mg.

Case 58. Man, aged 31. Acute nephritis twelve years ago. Good health until uraemic restlessness and pulmonary oedema developed in last four weeks. Lungs very oedematous, with great dyspnoea and distress, though semi-comatose. Blood pressure 200 mm. Heart 18 oz. No atheroma. Small red kidneys.

Glands 12 hrs. post-mortem, 7·1 grm., fat full, small adenomata.

7·5 grm. = 2·1 mg.; the increased weight of the glands was largely due to oedema.

Case 59. Man, aged 42. Polyuria for twenty years. Uraemia with vomiting for six months. Ascites. Blood pressure 180 to 140 mm. Pericarditis and slight fever. Heart 21 oz. Very slight atheroma. Congenital hydronephrosis.

Glands 8 hrs. post-mortem, full of fat; no adenomata.

5·25 grm. = 3·5 mg.
5·55 grm. = 3·2 mg.

Case 60. Man, aged 30. Uraemic vomiting and headaches for ten months; later retinitis and fits. Blood pressure 200 mm. Temperature 98°, despite pericarditis. Red, fibrosed kidneys. Heart 21 oz. Very slight atheroma.

Glands 16 hrs. post-mortem, 6·65 grm., full of fat and small adenomata.

9·63 grm. with very large cortical adenomata = 3·1 mg. adrenalin.

Case 61. Boy, aged 16. Chronic nephritis for five years. Blood pressure 100 mm. Death by chronic uraemia with drowsiness. Fibrosed kidneys 1½ oz. Heart 7½ oz. No atheroma.

Glands 18 hrs. post-mortem, 4·72 and 4·05 grm., full of lipoid; no autolysis.

This slight enlargement of the adrenals had not caused any precocious growth of the boy, for he had an infantile appearance of 12 years old, such as is frequently seen in children with chronic kidney disease.

In nine other cases with high blood pressure similar results were found, the glands being sometimes a little enlarged by cortical adenomata, but otherwise normal in their contents. The presence of the adenomata was not directly related to the extent of atheroma in the aorta.

Secondary infections with fever brought with them the usual result, partial loss of the cortical lipoid, just as it would in the absence of kidney disease.

Case 62. Woman, aged 37. Uraemic headaches for six months. Blood pressure 190 mm. Death by respiratory obstruction from rapidly spreading impetiginous infection of nose and face. Temperature 100°. Small, white granular kidneys.

Glands 3 hrs. post-mortem, 4·5 grm., fat considerably exhausted.

2·53 grm. with irregular areas of old cortical atrophy. No adenomata. No increase of medulla.

B. Chronic parenchymatous nephritis and large smooth kidneys. There were four cases, and the results were identical with those of interstitial nephritis.

Case 63. Man, aged 33. Albuminuria and dropsy three years, following acute nephritis. Uraemia last six months. Blood pressure 180 mm. Comatose at death. Heart 18 oz. A little yellow atheroma. Large smooth kidneys.

Glands 20 hrs. post-mortem, lipoid fair; medulla full size.

5·1 grm.
3·97 grm.

Case 64. Man, aged 30. Oedema and headaches, following acute nephritis one year ago. Retinitis. Blood pressure 200 mm. Large white kidneys. Heart 14 oz. No atheroma.

Glands 18 hrs. post-mortem, lipoid abundant and small adenomata.

5·46 grm. = 5 mg.

Case 65. Man, aged 41. Bright's disease 7½ years ago. Oedema three months. Blood pressure 150 mm.; pericarditis and slight broncho-pneumonia. Temperature 99°. Slight atheroma. Heart 21 oz. Kidneys fibrosed and small, 3½ oz.

Glands 20 hrs. post-mortem, no adenomata, fat full.

4·88 grm. = 2·45 mg.
3·41 grm. = 1·9 mg.

Summary.

From all this tedious detail emerges at any rate one generalization, that the cortical lipoid is stored and consumed under conditions entirely different from those which control the body fat in general. The starvation of cancer, diabetes, and especially of anorexia nervosa strips the fat from the body: it leaves unchanged the lipoid of the adrenal cortex. Pathological intoxications, such as that in diphtheria, increase the apparent fat in the heart and kidney: they only exhaust the cortical lipoid. Except in the degenerating areas of the foetal cortex there is no abnormal accumulation of fat in the adrenal under ordinary conditions of disease. On the contrary, there is actual loss. The lipoid vanishes with great rapidity in acute septic fevers, slowly in chronic fevers; and it is also diminished in severe haemorrhage and anaemias.

The load of lipoid is not directly related to that of adrenalin. It is true that both are reduced in septic fevers, but there are several conditions in which the adrenalin is lessened without any change in the lipoid. The cleanest example of this was in Cases 7 and 8, where circulatory failure and mental distress had exhausted the medulla, yet the cortical fat seemed normal. So, too, the adrenalin is lessened by the strain of a surgical operation, which leaves the lipoid unaffected. In my experiments on cats, exhaustion of the adrenalin was produced in various ways; in none of these was any alteration detected in the cortical fat, and there was no suggestion that the latter was controlled, like the adrenalin in the medullary cells, by the splanchnic nerves.

The nature of this loss of cortical lipoid cannot be explained by the evidence at present available.

1. It might be that the mere rise of temperature in the fevers carries it

away by a change of physical state. Such non-septic hyperpyrexia as occurs in some fatal cerebral haemorrhages would be of great value in answering this question as to whether the loss is caused by poisoning or simply by the rise of temperature. I have not had the opportunity for such an analysis apart from the exhausting complications introduced by broncho-pneumonia. But Case 45, where a final temperature of 107° was reached after a cranial operation, comes near to the desired condition, and in it the fat was normal. Experimentally I have compared the glands in cats under ether, of which the one was cooled to 96°, rectal temperature, and the other heated to 107° (normal 100° to 102°) for three hours. No alteration could be detected in the adrenal lipoid. Hence it does not seem probable that this simple physical change suffices to explain the disappearance of the lipoid.

2. A second suggestion is that the cortex is concerned in upholding the cholesterin ester content of the blood, and that the latter may play an essential part in resisting the poisonous effect of the bacterial infections, while its excess may lead to atheromatous changes in the walls of the blood-vessels. Workers abroad have shown that the cholesterin percentage in the blood sinks in fevers. Some such relationship would also serve to explain the frequent diminution of the adrenal lipoid in severe haemorrhages, when these substances may be lost to the body with the escape of the blood. There is no satisfactory experimental evidence on this point. Gardner and Lander (24), by chemical analyses in starved cats, found that the cholesterol of the adrenals fell during starvation, while that of the blood was still upheld. Weltmann was inclined to think that infections in guinea-pigs caused at first an actual increase in the lipoid, and that exhaustion followed rapidly as the intoxication increased.

On the other hand, the especial growth of the cortex in the human foetus—provided that the cerebral hemispheres of the child are developing to their full measure—and its partial atrophy after birth, just when the infant is exposed to a world of new bacterial infections, seem totally opposed to any theory which would connect the lipoid with the febrile reactions of septic infection.

3. Bodily growth and sexual maturation are undoubtedly controlled to some extent by the adrenal cortex. The evidence reviewed by Glynn (25) is conclusive on this point. Morphologically the cortical cells are related to the interstitial cells of the generative glands, upon which in the testicle the male characteristics of the body depend. The cells in the testis, like the lutein cells of the ovary, contain a lipoid closely resembling that of the adrenal. But it is obvious that this fatty substance is unlikely to be the material by which sexual maturation is effected, if it can disappear in the course of a couple of days' fever. And it must be remembered that the lipoid is not present in the adrenals of all mammals, nor always in the testicles. The growth function is probably to be ascribed to some other secretion of the cells than the lipoid. Curiously, the fat in the sex glands was found to show changes closely parallel to that of the adrenal cortex. Thus it was abundant in the lutein cells of the emaciated woman (Case 4); it was lost in the febrile disease of Case 29, and moderately

exhausted in Case 79. Similar changes were seen in the testicles, though the comparison was not analysed with sufficient detail.

I have not seen any examples of sexual precocity associated with cortical tumours, and no pathologist, into whose hands such material has fallen, has, so far as I am aware, considered the presence or absence of lipoid in the growth. Hypernephromata arising in the kidney in some instances do secrete a lipoid. In two cases examined by me, the lipoid was also secreted by the cells in the secondary deposits elsewhere, yet the patients exhibited no special symptoms that could be ascribed to this material, and no atheromatous changes were found in their aortae.

Adenomata are common in the cortex, and they are always abundantly laden with lipoid. Their cells, though well vascularized, do not part with their fat so quickly as does the normal cortex in fever, a fact which suggests that there may be some obscure nervous control of the cortical cells. They arise chiefly from the cortical islets embedded in the medulla, but they do not cause pressure symptoms, as do those of the thyroid or pituitary; and it cannot be said that their presence is a mark of special disease and an index that the gland's functions have of late been either exalted or depressed. In five of the cases quoted in this paper the adenomata were very large, so much so as to double the weight of the gland. Two were tuberculous patients, but this seemed to be only a chance association. One was from chronic renal disease.

Small, multiple adenomata of the cortex are very frequent, and they do tend to appear with greater frequency in renal cases, though Cases 53, 54, 59, and 62 show that the phenomena of kidney disease and high blood pressure could be developed for a long time without the growth of these nodules. The relationship of the cortical lipoid to renal disease and to atheroma is discussed later.

ADRENALIN.

The results of the analyses for adrenalin may be briefly recapitulated. As stated earlier, the normal gland of a healthy man weighs between 4 and 5 grm. and yields from 4 to 5 mg. of adrenalin by my method of assay.

Children. I have not been able to collect material enough to show to what extent the adrenalin load alters at different ages of life. Schmorl and Ingier stated it to be practically uniform for ages from 10 to 40; with a slight falling off in old age. They found the average load for one gland at birth to be 0·08 mg., at 2 years 0·6 mg., at 5 years 1·5 mg., at 10 and onwards 2·0 mg. Their figures probably record not more than half the real load, though they may be enough for comparison relatively to one another. Goldzieher gives the figures 0·5 mg. for the gland of a new-born child, 2 mg. for an adult; and he does not attempt to trace the increase of the load in early youth.

My own observations gave slightly different results. At birth the medulla of the adrenals is small, but there is a large mass of chromaffine cells outside the gland in the organ of Zuckerkandl, to which Kohn has given the better name of the paraganglion aorticum. Biedl and Wiesel (6) proved that this paraganglion contains adrenalin. I measured the total amount that could be extracted and found it to be very considerable, nearly 1 in 500 of the entire weight of the paraganglion. On the other hand, from the adrenal gland itself at birth only a trace of adrenalin could be obtained.

Case 66. Full-term child: transverse presentation. Death in delivery.
Gland 3 hrs. post-mortem, 3·21 grm. = 0·012 mg.
Entire paraganglion aorticum of both sides 0·1 grm. = 0·12 mg.

Case 67. Full-term child: breech presentation and death in delivery.
Gland 4 hrs. post-mortem, 2·7 grm. = 0·009 mg.
Entire paraganglion 0·11 grm. = 0·24 mg.

Case 68. Full-term child six days old. Normal delivery, but death from asphyxia in succession of fits. No cause of death found. The adrenals showed uniform enlargement of the cortex with no special features.
Gland 14 hrs. post-mortem, 5·72 grm. = 0 mg. adrenalin.
Kidney 15·52 grm.
Entire paraganglion 0·125 grm. = 0·225 mg.
Kidney and adrenal of other side 27·7 grm.

The weight of a normal adrenal at birth is slightly above 2 grm., and this figure is liable to be abnormally increased by congestion of the gland with blood when the death is asphyxial, as in Case 66. Consequently Case 68 was at least double the normal size. No special features, except the unexplained fits, could be found in connexion with this hypertrophy.

These observations show that the adrenal just before birth is in no respects comparable with the gland of post-natal life. The foetal cortex degenerates after birth, and is supplanted by a fresh growth (19). The foetal medulla before birth contains practically no adrenalin, for the latter is provided by the chromaffine tissues outside the gland in the close neighbourhood of the sympathetic ganglia; and these chromaffine masses, like the foetal cortex, degenerate soon after the child is born, when the medulla begins to assume its proper function.

I have no figures for the glands of children in health. Those in disease may be quoted here, but it should be remembered that they are all under conditions leading to partial exhaustion.

Case 16. 2 years and 8 months. Diarrhoea 1 week.
Gland 12 hrs. post-mortem, 1·62 grm. = 0·31 mg.

Case 14. 2 years and 8 months. Post-diphtheritic paralysis of diaphragm; asphyxia and broncho-pneumonia.
Gland 4 hrs. post-mortem, 1·56 grm. = 0·7 mg.

Case 15. 3½ years. Haemorrhagic diphtheria 2 days.
Gland 17 hrs. post-mortem, 1·4 grm. = 0·37 mg.

Case 69. Child 5½ years. Toxic diphtheria.
Gland 32 hrs. post-mortem, 2·2 grm. = 0·55 mg.

Case 46. Girl, 13 years. Death after 2 hours' operation under chloroform.
Glands 18 hrs. post-mortem, $\frac{2 \cdot 65 \text{ grm.}}{2 \cdot 46 \text{ grm.}} = 1 \cdot 8$ mg.

Case 19. Boy, 13 years. Chronic septic meningitis for 5 weeks.
Glands 5 hrs. post-mortem, $\frac{4 \cdot 75 \text{ grm.}}{4 \cdot 52 \text{ grm.}} = 2 \cdot 0$ mg.

These figures give no reliable indication of the rate at which the glands grow, nor of the age when they attain their adult load of adrenalin.

Exhausting Conditions.

Fright, anaesthetics, cerebral injury with reactive rise of blood pressure, excitation of afferent nerves, and bacterial intoxications have been proved by my experiments on cats to exhaust the residual adrenalin through the splanchnic nervous control of the glands.

The following examples will illustrate the extent of this loss and the rapidity with which it occurs. In each experiment the splanchnic nerve to the gland was cut on one side. By this section one gland was isolated from the exhausting influences, and so kept in a resting state to serve as a measure of the original load of adrenalin at the time when the experiment was begun.

Ether 6 hours: resting gland 0·26 mg.; exhausted 0·07 mg.

Chloroform 5½ hrs.: resting 0·21 mg.: exhausted 0·08 mg.

Cerebral haemorrhage and destruction of brain 6 hrs.: resting 0·19 mg.; exhausted 0·03 mg.

Morphia 6 hrs., the drug being merely excitant to cats: resting 0·22 mg.; exhausted 0·07 mg.

It was also proved that cats which had been recently admitted to the laboratory and were alarmed and suspicious of their new surroundings always had much less adrenalin in their glands than those which had been in a longer time and become happy and contented. These factors, which lead to quick loss of adrenalin in a cat, must certainly act in man also. They need consequently to be considered in each separate case with especial reference to the conditions that immediately preceded death, before any conclusions can be drawn with regard to the general exhausting influences of the disease that led to death. Moreover, the analyses quoted in the earlier pages of this paper show that after death there is a steady loss of adrenalin by autolytic changes, which increases hour by hour and in twenty hours may amount to 15 or 20 per cent. of the total. This also must be taken into consideration, so that it becomes necessary to name for each case the time that elapsed post-mortem before the glands were extracted for analysis. With all these complex conditions affecting human pathological material, one is therefore compelled to adopt the tedious method of quoting each separate case in detail, so that each may be judged individually with regard to these details. It is quite misleading to calculate averages from the figures given; nor can the examples quoted be tabulated in columns for a comparison except of the roughest nature.

Effect of Anaesthesia.

Case 46. Girl, aged 13. Normal health. Chloroform for 2 hours.
Gland 18 hrs. post-mortem, 2·65 grm. = 1·8 mg.

Case 51. Man, aged 42. Acromegaly, chloroform for 3 hours.
Gland 15 hrs. post-mortem, 7·83 grm. = 4·2 mg.

Case 45. Woman, aged 36. Cerebellar decompression under open ether. Death 15 hours later.
Gland 12 hrs. post-mortem, 3·63 grm. = 1·5 mg.

Case 43. Man, aged 57. Death $1\frac{1}{2}$ hours after extensive operation for removal of glands from the neck, which lasted 3 hours under intravenous ether.
Gland 5 hrs. post-mortem, 3·59 grm. = 3·6 mg.
3·7 grm. = 3·6 mg.

Case 44. Woman, aged 47. Death 8 hours after operation for removal of vagina, which lasted 45 minutes under intravenous ether.
Gland 8 hrs. post-mortem, 4·76 grm. = 3·2 mg.

These examples suggest a loss of adrenalin under anaesthetics, varying from 20 to 50 per cent. and insufficient to cause death. The differences in the figures in each case illustrate the difficulty of deduction from human material, and in themselves would hardly have been enough to justify the belief that anaesthetics do exhaust the medulla. In the cat the result was quite clear, because the gland of one side taken out at the commencement of anaesthesia could be contrasted with the other, which was removed at the end of the experiment. Under these experimental conditions the adrenalin load was reduced by 50 per cent. when ether or chloroform had been administered for four or five hours.

Cerebral Compression.

Here it is necessary to distinguish two types. First come those cases, as in a cerebral haemorrhage, where the rise of pressure is sudden and the vasomotor centres in the medulla react energetically to produce the characteristic slow pulse, and the rise of blood pressure with presumably a simultaneous call upon the adrenals. These conditions were proved to exhaust the adrenalin in cats. But in the second group, as especially with tumours and spreading oedema of the brain, there is more often a progressive coma that deepens into a quiet sleep and so reaches the end without any emphatic features of bulbar excitation. In these the adrenals show little loss.

Case 70. Man, aged 49. Sudden and extensive pontine haemorrhage; death in eleven hours. For the first six hours the blood pressure was 250 and temperature 100°; then it sank to 130, and so progressively to death. Heart 16½ oz.; kidneys slightly fibrosed.

Glands 12 hrs. post-mortem, 4·4 grm. = 1·2 mg.
4·3 grm. = 1·36 mg. fat normal; no adenomata, and medulla certainly not enlarged.

Case 71. Woman, aged 49. Extensive haemorrhage in right external capsule which slowly forced its way inwards to the ventricles. She rapidly became unconscious and died in ten hours. Blood pressure 230–40, and axillary temperature 104·6°. Kidneys slightly fibrosed; heart 10 oz., and slight atheroma of aorta.

Glands: Left 5 hrs. post-mortem, 4·22 grm. = 1·12 mg.
Right 10 hrs. post-mortem, 4·38 grm. = 1·0 mg. No autolysis, fat full, medulla small.

These were undoubted examples of exhaustion in the attempt made by the vaso-constrictor centre to sustain the pressure and the cerebral circulation.

Case 72. Man, aged 56. Post-parietal glio-sarcoma, with headache and vomiting for one month before death; fair recovery; and then rapidly deepening coma in last thirty hours with slight left hemiplegia. Final blood pressure not measured.

Glands: Left 2 hrs. post-mortem, 2·82 grm. = 0·9 mg., being irregularly atrophied.
Right 22 hrs. post-mortem, 6·65 grm. = 1·25 mg., fat full, and no autolysis.

Case 25. Man, aged 55. Pachymeningitis haemorrhagica; two days' coma. Blood pressure 160 and terminal fall.

Glands 6 hrs. post-mortem, 4·95 grm. = 3·0 mg.
4·85 grm.

Case 73. Man, aged 61. Large cystic frontal glioma. Ill for four months with apraxia and recurrent cerebral coma. No vomiting. Blood pressure 140–60. Became very emaciated towards end.

Glands 2 hrs. post-mortem, 4·27 grm. = 4·0 mg.
4 hrs. post-mortem, 5·68 grm. = 4·0 mg.

Case 74. Woman, aged 32. Glio-sarcoma of temporo-sphenoidal lobe, causing death by cerebral compression. Slow development of quiet coma resembling sleep. Blood pressure normal at 120. Recovered full consciousness after parietal decompression, but died suddenly thirty-six hours after.

Glands 7 hrs. post-mortem, 5·45 grm. = 2·4 mg.
5·9 grm. = 2·5 mg.

Here the anaesthetic and the operation complicated the result and were in themselves partly responsible for the moderate loss of adrenalin. There was no rise of blood pressure to exhaust the glands.

Heart Failure.

Case 75. Man, aged 46. Old mitral regurgitation with much oedema and ascites for nine weeks. Auricular fibrillation. Sudden death when commencing to improve under digitalis. Heart much enlarged.

Gland 15 hrs. post-mortem, 3·35 grm. = 3·6 mg.

Case 76. Woman, aged 21. Old aortic regurgitation and anginal attacks for years. No oedema; well nourished. Sudden death after two or three weeks of recurrent pain and moderate distress. Heart 27 oz.

Glands: Left 2 hrs. post-mortem, 3·1 grm. = 3·2 mg.
Right 20 hrs. post-mortem, 4·45 grm. = 3·25 mg., enlargement of this gland being chiefly by cortical adenomata.
Assay of old extract of left gland, after standing 18 hrs. = 3·0 mg.

This may be accepted as the actual true load in the course of chronic heart disease, insomuch as death occurred suddenly. But in the next two instances of acute heart failure, the sufferer was in obvious mental distress for the two or three days preceding death, and though the blood pressure was low and the pulse was feeble, the vasomotor nerves were doubtless strained to their uttermost to help the dying heart. Observations on the effect of fright in exhausting the adrenalin in cats emphasize the need to realize how rapidly the glands may be emptied in man during emotional anxiety and distress.

Case 7. Girl, aged 16. Chronic valvular disease. Acute collapse with great mental distress in last thirty hours.

Glands 9 hrs. post-mortem, 4·35 grm. = 0·7 mg.
4·75 grm. = 0·7 mg.

Case 8. Woman, aged 26. Three years' illness from hypertrophied feeble heart. Complete cardiac failure in last few days with much distress.

Gland 8 hrs. post-mortem, 4·7 grm. = 0·2 mg.

On the other hand the next case showed little loss of adrenalin, despite the cold skin and feeble circulation, presumably because the patient was apathetic and reactionless.

Case 9. Man, aged 35. Mitral stenosis and auricular fibrillation. Death by slowly progressive thrombosis within auricles. No mental distress.

Glands 2 hrs. post-mortem, 5·42 grm. = 2·3 mg.
5·78 grm. = 2·3 mg.

Malignant Endocarditis.

Heart failure is added to septicaemia, but the latter by its intoxication is the chief cause of death. The adrenalin is exhausted, but not so much as is the lipoid; whereas in the preceding cases the lipoid was not affected at all.

Case 23. Gland 18 hrs. post-mortem, 5·39 grm. = 0·75 mg.
Case 22. Glands 20 „ „ 6·3 grm. = 0·75 mg.
4·7 grm. = 0·35 mg.
Case 20. Gland 13 „ „ 6·47 grm. = 2·1 mg.
Case 21. „ 8 „ „ 3·72 grm. = 2·3 mg.

Case 77. Woman, aged 48. Old rheumatic heart. Malignant endocarditis for five weeks, with many streptococci in blood and fever, 101° to 103°.

Gland 24 hrs. post-mortem, 4·5 grm. = 1·5 mg.

Pneumonia.

Case 10. Man, aged 37. Acute pneumonia three days.

Gland 5 hrs. post-mortem, 10·35 grm. = 1·45 mg.

Case 11. Man, aged 42. Pneumonia seven days.

Gland 22 hrs. post-mortem, 7·4 grm. = 1.2 mg.

Case 13. Man, aged 42. Pneumonia six days, and old syphilitic aortic disease.

Glands 4 hrs. post-mortem, 7·23 grm. = 2·5 mg.
6·72 grm. = 2·5 mg.

Other Septic Infections.

Case 18. Man, aged 29. Perinephritic abscess and pyaemia.

Gland 20 hrs. post-mortem, 7·72 grm. = 2·5 mg.

Case 78. Man, aged 26. Gangrenous broncho-pneumonia and empyema for four weeks. Temperature 100° to 103°.

Gland 18 hrs. post-mortem, 5·6 grm. = 1·75 mg.

Case 79. Woman, aged 18. Small chronic empyema for five years, but otherwise in good health. Estlander operation was followed by death seventeen days later with abscesses in chest wall, in occipital lobe of brain, and with pus in ventricles. Fever 101° to 104°.

Glands 18 hrs. post-mortem, 7.98 grm. = 1·4 mg.
5·63 grm. = 1.25 mg. moderate fat in cortex, and also in corpora lutea.

Case 80. Man, aged 48. Closed abscess in lung with rigors and high fever in last week. Haemorrhage from old gastric ulcer. In final twenty-four hours temperature rose from 105° to death at 106°.

Glands 9 hrs. post-mortem, 4·59 grm. = 2·6 mg.
5·68 grm. = 2·5 mg.

In all these septic states the weight of the gland tended to be increased and the lipoid diminished ; and in all there was some exhaustion of the adrenalin, though not so great as in the case of cardiac failure, and probably not so great as in itself to endanger life.

Tuberculosis.

Case 35. Man, aged 29. Addison's disease ; no fever.

Gland 8 hrs. post-mortem, 10·1 grm. = 0·03 mg.

Case 36. Man, aged 34. Chronic Addison's disease for ten years. Atrophied tuberculous glands.

Gland 6 hrs. post-mortem, = 0 mg.

Case 33. Man, aged 28. Miliary tuberculosis.

Gland 24 hrs. post-mortem, 7·8 grm. = 1·4 mg.

Case 32. Woman, aged 45. Pulmonary and intestinal tuberculosis.

Gland 8 hrs. post-mortem, 8·8 grm. = 2·14 mg.

Case 81. Woman, aged 35. Chronic tuberculous salpingitis and enteritis with miliary tuberculosis of lungs and peritoneum. Temperature 101° and 103°.

Glands 7 hrs. post-mortem, 5·23 grm. = 1·2 mg.
6·08 grm. = 1·2 mg., large tuberculous areas.

Anaemia.

Case 41. Woman, aged 33. Death caused in nineteen days by haemorrhage from gastric ulcer, and this was associated with mild malignant endocarditis.

Gland 7 hrs. post-mortem, 6·7 grm. = 3·25 mg.

Case 42. Man, aged 30. Splenic anaemia with repeated haemorrhages.

Gland 8 hrs. post-mortem, 4·26 grm. = 2·5 mg.

Case 82. Woman, aged 34. Multipara. Severe ante-partum haemorrhage in eighth month. Bleeding for four hours; cervix dilated and child delivered under chloroform; patient died two hours later from shock and tear of vagina.

Glands: Right 4 hrs. post-mortem, 4·5 grm. = 1·47 mg.
Left 21 hrs. post-mortem, 5·92 grm. = 2·8 mg., fat very abundant.

Case 83. Woman, aged 24. Chronic ulcerative colitis. Haemorrhage in last few days but not of a serious nature; sudden death by pulmonary embolism. No fever.

Glands 7 hrs. post-mortem, 4·14 grm. = 2·0 mg.
4·55 grm. = 2·1 mg., fat full.

Loss of blood seems to affect the adrenalin much less than might have been anticipated from the disturbances brought about at the same time in the vascular adjustments.

Other Conditions.

Case 5. Man, aged 39. Ulcerative colitis and extreme emaciation. No fever; death by diarrhoea and exhaustion.

Gland 10 hrs. post-mortem, 4·8 grm. = 1·8 mg.

Case 4. Woman, aged 29. Anorexia nervosa with great emaciation. Final septic pneumonia for one week.

Glands 14 hrs. post-mortem, 5·82 grm. = 2·0 mg.
5·2 grm. = 2·0 mg.

Case 50. Man, aged 62. Diabetic coma.

Gland 8 hrs. post-mortem, 6.8 grm. = 2.0 mg.

Case 84. Man, aged 36. Tetanus with moderate locking of jaw, and marked contraction of trunk muscles for four days. Sweating with muscular strain, but no fever and no mental alarm. Died in sudden spasm during administration of chloroform. Both psoas muscles found to be ruptured.

Glands 12 hrs. post-mortem, 3·87 = 2·31 mg., fat normal.
3·88 = 2·31 mg.

Case 26. Woman, aged 42. Cancer of lung and thoracic effusions. Ill four months. Sudden death by pulmonary embolism.

Gland 18 hrs. post-mortem, 6·32 grm. = 4·2 mg.

Here again the figures are not very conclusive. The last case (26) shows that the invalid life entailed by cancer and some mechanical embarrassment of the heart and lungs does not of necessity reduce the adrenalin. But as a rule there is considerable exhaustion in all the septic illnesses, and especially so in acute miliary tuberculosis. Nothing can be said more definitely than this. Once more appears the contrast between the cortical lipoid and the adrenalin. The fat was abundant in Case 5, where the adrenalin was low; there was hardly any in Case 41, where the adrenalin was, for illness, high.

Mott and Halliburton thought that the adrenalin was especially reduced in chronic disease, such as tuberculosis, cancer, or chronic Bright's disease, whereas a good reaction was found by them in pneumonia. This can hardly be regarded as other than a mistake. It is acute febrile illness which especially exhausts both the cortex and the medulla of the gland: the chronic ailments depreciate the stores to a much less serious degree.

For convenience I have tabulated some of my analyses in a series of what seemed to me typical cases. Each is an individual analysis, for, as was urged earlier, the conditions before and after death varied so widely in the several examples of each particular disease that the calculation of an average figure would not represent the change with any greater accuracy. So many slight factors affect the load of adrenalin found by assay that it is unwise to notice any difference of less than 0·5 mg., and figures in the second place of decimals are not worth recording.

TABLE I.

	Cause of Death.	Weight of Gland.	Adrenalin.	Lipoid.
Child.		grm.	mg.	
Full term	Asphyxial	2·5	0·01	slight
	Paraganglion	0·11	0·22	nil
$2\frac{8}{12}$ yrs.	Post-diphtheritic asphyxia	1·6	+0·7	
$3\frac{1}{2}$ yrs.	Haemorrhagic diphtheria	1·4	+0·4	moderate
13 yrs.	Chloroform and shock	2·5	1·8	,,
Adult.				
	Normal. Accident	4	4·5	full
	Surgical shock	3·7	3·6	,,
	Long anaesthesia	—	2 to 3·0	,,
	Cerebral tumour and coma	4·2	4·0	,,
	Cerebral haemorrhage with raised blood pressure	4·4	1·3	,,
	Cancer: sudden death	6·3	4·2	,,
	Heart disease:			
	sudden death	3·4	3·6	,,
	slow failure and apathetic death	5·5	2·3	,,
	acute failure and mental distress	4·5	0·2 to 0·7	,,
	with septicaemia of malignant endocarditis	4 to 6	0·7 to 2·0	lost or slight
	Haemorrhage from:			
	splenic anaemia	4·2	2·5	full
	gastric ulcer	×	×	slight
	Pneumonia:			
	acute	10·35	1·45	lost
	slow	7·0	2·5	slight
	Pyaemia	7·7	2·5	lost

Table I (*continued*).

Cause of Death.	Weight of Gland.	Adrenalin.	Lipoid.
Adult (continued)	grm.	mg.	
Tuberculosis:			
Addison's	10·1	0·3	traces
miliary	7·8	1·4	moderate
acute pulmonary	8·8	2·14	slight
Nephritis:			
parenchymatous	4·8	2·4	full
chronic interstitial:			
uraemia	4·4	—	,,
with pericarditis	5·3	3·5	,,
arterio-sclerotic	7·0	4·3	,,
Acromegaly	7·83	4·2	moderate

Schmorl and Ingier's tabulated results differ from mine, partly because their standard of health, 2·33 mg., was so much lower than what I found, namely 4 mg. Measuring from this standard they record:

A slight increase for pneumonia	2·44 mg.
Slight decrease for septicaemia and malignant endocarditis	2·28 ,,
Greater decrease for miliary tuberculosis	2·03 ,,
Increase in acute nephritis	3·01 ,,
Increase in interstitial nephritis with large heart	3·25 ,,
Increase in valvular heart disease	3·27 ,,

The authors dealt with their results with caution, pointing out the difficulties of drawing any deductions from these figures.

Goldzieher's conclusions agree more closely with mine. His standard for one gland of a healthy adult was 2 mg. In septic conditions this fell to 0·75, while the gland became swollen and the cortical lipoid was observed to disappear. For chronic nephritis and arterio-sclerosis he found the contents to be 2·9 mg., a slight increase under conditions of high blood pressure which agrees with Schmorl and Ingier but is at variance with the observations that I describe subsequently.

High Blood Pressure and Athero-sclerosis.

In discussing these diseased states there are two separate and distinct theories to be considered, both of which have been developed chiefly by French thought. Shortly after the discovery of the intense vaso-constriction caused by extracts of the medulla, it was naturally suggested that persistently high blood pressure might be explained by hypertrophy of the medulla and too abundant secretion of adrenalin. Adrenalin was later proved to cause atheroma in the aorta when injected repeatedly into the blood-stream; and this observation at once made it possible to ascribe both the high pressure and the atheroma to one and the same cause, excess of adrenalin in the blood from a supposed hypertrophy of the medulla. The theory was of course followed by the statement that the medulla is hypertrophied in such conditions.

The second view is of later origin, and it looks for evidence of disease only in the cortex of the gland, neglecting the problem of the rise of blood pressure. Damage of the kidney makes it impermeable to certain substances which are consequently retained in the body. The lipoids of the cholesterin ester group may under these conditions appear in excess in the blood and be later deposited in various places in the body,[11] much as urates are in the uricaemia of gout. Atheromatous changes in the aorta, and excess of lipoid in the adrenal together with cortical adenomata, would both be visible signs of such a state; and atheroma would then be especially associated with excess of cortical lipoid. Chauffard (13) has demonstrated an increase of cholesterin in the serum of patients with nephritis and arterio-sclerosis (0·27 per cent. to 0·42 per cent.) and correlated this with a high cholesterin percentage in the adrenals. On the other hand, the proved loss of the cortical lipoid in acute septic infections would, in the same way, be harmonized with the observations of Chauffard that the percentage of cholesterin in the blood (0·15 to 0·18 per cent.) sinks in the acute septic fevers, and particularly so in acute pneumonia.[12]

The facts are equivocal with regard to the second view, and they almost exclude the first.

Three measurements are needed to justify the statement that the medulla is overactive in a particular case:

It is necessary to know: (1) The percentage of adrenalin circulating in the blood; (2) the amount of adrenalin that can be extracted from the gland after death; (3) the actual bulk of the medullary tissue.

The argument would be good, if an increase could be demonstrated in all of these three factors. So far as I am aware, it has as yet been proved in none. Stewart's (41) criticisms show that at present no reliable method has been found which proves the presence of adrenalin in the general current of the blood. The substance is easily recognized in the blood of the adrenal vein, but it disappears rapidly from the blood during circulation through other tissues, and consequently it cannot be demonstrated with certainty in the superficial veins of the body, which alone are accessible to clinical study. Borberg (8, p. 399) failed to find any action on the frog's pupil with the blood from cases of nephritis or exophthalmic goitre. Fränkel (23), using the rabbit's uterus as a test organ, also found no increase of adrenalin in the centrifugalized serum of chronic nephritis, whereas in exophthalmic goitre he recorded an increase of from four- to eightfold. In the blood of health he found the total amount of adrenalin to be 12·5 mg., but 50 to 100 mg. in the goitrous disease. These figures are very difficult to accept, considering that the entire store of adrenalin in the two glands in health is only about 10 mg. It is therefore clear that statements with regard to the measurement of (1) are at present to be received with criticism and reserve. The theory does not decide whether the cholesterin bodies are first formed in the adrenal cortex or absorbed by these cells from the excess in the blood-stream. (2) can be determined with fair accuracy. The figures given in this paper show that the residual adrenalin in cases of persistently high blood pressure is of practically the same amount as that seen in death from other diseases. (3) offers a laborious task. The medulla is aggregated chiefly at the venous pole of the gland, in a thick mass from which the laminar extension between the cortex of the triradiate tail is very thin and irregular. A series of sections transversely to the venous axis from pole to pole of the gland are therefore needed for a fair estimate of the bulk of the medulla. Longitudinal sections are very misleading. Accurate measurements of the total bulk of the tissue could be obtained by adding up the areas of all the sections, as was done by Tuckett and myself in the glands of some of the smaller animals (20, p. 339). It seemed an extravagance of labour to attempt this in the human gland, and I was content with the rough naked-eye estimate made by looking at a series of slices.

---

[11] Experiments have been made in St. Petersburg (1 and 14) and in Munich (46) which illustrate the deposition of cholesterin in various tissues of the body, when rabbits were fed with this substance for a long time.

[12] Picard (38) has moreover shown in dogs that the cholesterin of the blood and of the adrenal cortex alike may be lessened by prolonged exercise.

Obviously it cannot be admitted that the medulla must be enlarged because the gland's weight is increased. Oedema in the last few days of life may double its weight, as conspicuously in the cases of acute pneumonia already quoted. Moreover, the cortex forms probably 80 to 90 per cent. of the entire bulk of the gland,[13] and it is very prone to increase of mass by irregular adenomata, and even perhaps to alteration of its weight by the variations that occur in the load of lipoid. A relatively small change in the cortex would add 1 grm. to the total weight of the gland, whereas an increase of 1 grm. in the medulla would mean that the latter was almost trebled in bulk with the possibility of a similar trebling of its adrenalin load.

This difficulty has been neglected by several writers, who have sought for evidence that high blood pressure in renal disease must be associated with hypertrophy of the adrenal medulla, and being perhaps too eager to believe in the theory were reluctant to criticize the facts. The original observations on this question in France were made by Pilliat (1903) and Vaquez, but most of the French workers since then have abandoned the view. In Vienna it is still upheld by Neusser and Wiesel (1910) upon evidence that is in nowise convincing. Wiesel (49) stated that in chronic renal cases with cardiac hypertrophy the medulla is seen in section to be enlarged. But he does not describe the plane of the section with reference to the varying distribution of the medulla, nor does he give the total weight of the glands, nor measure the amount of residual adrenalin. Further, he admits that such hypertrophy is not always seen in chronic nephritis, and that it is not invariably related to high blood pressure, for he saw it in cases of nephritis where the blood pressure was not raised (35, p. 86).

Philpot (37) weighed the glands of twenty-seven cases, of which nineteen were examples of chronic interstitial nephritis. The average weight of each gland in the high pressure series was roughly 6·0 grm., and rather less than 4 grm.

---

[13] I am not aware of any actual measurements of this ratio in the human adrenal. Tuckett and I found that the cortex formed 98 per cent. of the gland in the guinea-pig, 95 per cent. in the rat, 90 per cent. in the rabbit and cat, 85 per cent. in the dog, and only 45 per cent. in the fowl. If similar proportions hold for man, an average healthy gland weighing 4·5 grm. will contain only 0·5 grm. medulla, the latter yielding 4 to 5 mg. adrenalin, or about 1 per cent. of its own weight.

in the others: on section he averred that it was the medulla which showed the hypertrophy and not the cortex. The distinction was not proved. In some of his cases circulatory oedema or septic conditions may have been responsible for a temporary increase of weight of the entire gland; and indeed his evidence is of very equivocal value.

Borberg (9) could find no clear evidence for such hypertrophy of the medulla in man; and Bittorf (7), in definite contradiction of Wiesel, stated that the adrenals may be notably small in kidney disease with high blood pressure. It is true that Schmorl and Ingier, who were prevented by their method of analysis from weighing the glands, found an increase of the residual adrenalin in cases of acute or chronic kidney disease and also of valvular heart disease; but the increase was in their opinion too uncertain to be regarded as proof of a special connexion with athero-sclerosis or high blood pressure. Goldzieher also recorded a slight increase under like conditions.

Owing to the number of causes that may affect the weight or the adrenalin content of the glands, it is desirable to approach the question from the other side; not to seek for examples in which the gland is enlarged and there is also a high blood pressure, but to examine the cases in which with high blood pressure and chronic renal disease the patient dies, slowly or quickly, from simple uraemic intoxication, without any phenomena of secondary infections or circulatory failure. This is the pure form of the disease. If in them the adrenals consistently show no hypertrophy, and no accumulation of adrenalin, then it is reasonable to conclude that medullary hypertrophy does not play the main part in the malady, and that such enlargement as may at times occur in complicated cases is accidental or due to such complications.

I have already given in this paper examples of high blood pressure and renal disease of this clean type. The glands were not enlarged. For comparison, the list is summarized here in tabular form. (See p. 86.) Case 53 was a very good instance, the disease having existed for two years, and being closely watched for the last year (blood pressure 230 to 200); yet the glands were in all respects normal. Whenever the gland was heavier than usual, the increase could be attributed to adenomata of the cortex or to oedema; and in most cases the medulla looked, if anything, rather small on section. In Case 57 it was exceptionally small in bulk and gave a surprisingly low yield of adrenalin. Case 64 was the only one in which the gland was enlarged together with increase of adrenalin; and in it unfortunately the second gland was damaged in removal, so that it could not be ascertained whether the second gland was abnormally small, like that in Case 72, a condition which would lead to compensatory hypertrophy in the gland analysed.

Case 62 suggests a line of argument which might weaken the deduction from these recorded facts. In it one gland was certainly atrophic. It might be urged that in all these high pressure cases the adrenals pass through a phase of abnormal activity, which is then succeeded by failure of power and shrinkage, changes that in themselves might help to determine the fatal issue of the disease.

TABLE II.

Case No.	Age.	Ill.	Blood Pressure.	Death.	Adrenals.	Cortex.	Medulla.	Kidneys.	Weight of Heart.	Aortic Atheroma.
					grm.				oz.	
53	51	2 years	200 230	uraemic coma, 7 days	4·5 4·2	no adenomata	not +	fibrosed 4 oz. each	17	well marked
54	68	1 year	— 180	sudden thrombosis basilar aneurism	3·21 3·2	no adenomata	large	fibrosed 5½ and 5 oz.	12	none
62	37	6 months	190	respiratory obstruction by cellulitis	4·5 2·53	no adenomata	not +	fibrosed 3 oz. each	11	very slight
65	41	3 months dropsy, nephritis 7 years ago	150	pericarditis	4·88 = 2·45 3·41 = 1·9	no adenomata	—	fibrosed 3½ oz.	21	slight
56	38	2 years	270	uraemic coma, 3 weeks	5·64 5·32	small adenomata	very small	fibrosed 2 oz.	13½	slight
59	42	6 months	140 180	pericarditis	5·25 = 3·5 5·55 = 3·2	no adenomata	—	congenital hydronephrosis	21	very slight
70	49	?	250	pontine haemorrhage, 11 hours	4·4 = 1·2 4·3 = 1·4	no adenomata	—	slight fibrosis 5 oz.	16½	very slight
71	49	?	230	capsular haemorrhage, 10 hours	4·22 = 1·12 4·38 = 1·08	very small adenomata	very small	slightly fibrosed 4 oz.	10	slight
60	30	10 months	200	uraemic fits	9·63 = 3·1 6·55	very large adenomata	—	fibrosed 3½ oz.	21	very slight
55	61	+ 1 year	200	arterio-sclerosis, mental failure	7·22 = 4·3 6·74 = 4·3	many small adenomata	—	arterio-sclerotic 4 oz.	22	very marked
58	31	Nephritis 12 years ago	200	pulmonary oedema, 4 weeks	7·5 = 2·1 7·1	oedematous small adenomata	—	fibrosed 3 oz.	18	none
57	50	2 years	170	9 days' drowsy uraemia	6·89 = 0·4 5·56 = 0·4	small adenomata	very small	fibrosed 2½ oz.	13	none
64	30	Dropsy 10 months	200	3 weeks' oedema and headaches	5·46 = 5·0 ? other	small adenomata	—	large white fibrosed 7 oz. and 6 oz.	14	none
63	33	Dropsy 3 years	180	3 months' headaches	5·1 3·97	no adenomata	large	large white 8 oz. each	18	very slight
61	16	Slight dropsy 5 years	100	uraemic coma	4·72 4·05	—	—	fibrosed 1½ oz.	7½	none

This hypothetical rise and fall would be analogous to the conditions of super- and sub-pituitarism, or to exophthalmic goitre succeeded by myxoedema. But clinically the possibility is almost denied by the fact that such patients do not in the last few weeks show a progressive fall of blood pressure. Moreover, in the case quoted, it was the cortex that was atrophied, not the medulla. In all the glands examined, though the medulla was often small, its cells looked healthy under the microscope.

Cerebral haemorrhage kills patients suddenly at the very height of disease, yet their glands are normal (Case 70, 4·4 grm. = 1·2 and 4·3 = 1·36 mg.; and Case 71, 4·22 = 1·12 and 4·38 = 1·08 mg.; partial exhaustion having been caused by the increased intracranial pressure). Hence the hypothesis, that the small size of the medulla and its apparently normal load of adrenalin are to be explained by terminal atrophy of a once exuberant mass of cells, is one that cannot be admitted. The observations given here show that in many cases of kidney disease with high pressure the glands are simply normal; in others they are enlarged by cortical adenomata. In none was there unequivocal increase of residual adrenalin; and yet the conditions of death, except in Cases 70 and 71, were not such as to exhaust the adrenalin.

The tendency towards cortical hypertrophy and the growth of small adenomata is a definite feature of kidney disease. In pure forms of renal fibrosis following old toxic influences, such as that of lead-poisoning, the adrenals may be of normal size, as in Case 53. But the arterio-sclerotic form of fibrosed kidney is particularly associated with enlargement of the adrenal cortex, witness Case 55. Between these are intermediate types; and the distinction is not a very clear one. Atheromatous changes in the aorta do not bear any constant relation to the blood pressure or to the hypertrophy of the adrenal cortex.

Changes in the adrenal cortex, in its bulk and in its load of lipoid, are the most manifest of the alterations wrought by disease, whether acute or chronic, in this gland. The medullary tissue, like the cells of the nervous system, seems to be less capable of overgrowth. It parts with its adrenalin under conditions which can be more or less understood by reference to the results of recent experimental work on animals; but there is no satisfactory proof that any form of disease is associated with an excess of such secretion. Hence the problem that must be solved and that seems to offer most in the reward of knowledge is the riddle of the cortical secretion. In this direction a step seems to have been made recently (45) by the isolation from the adrenal cortex of a vaso-constrictor substance akin to that found in defibrinated blood and differing from adrenalin.

Conclusions.

1. The normal adrenal gland of an adult man weighs between 4 and 5 grm. and contains from 4 to 5 mg. adrenalin.

2. There is no proof that the store of adrenalin in the medulla is increased in any disease.

3. The adrenalin is lessened in many infective diseases, but probably not to such a degree as to endanger the circulation.

4. The greatest loss was observed in examples of afebrile acute cardiac failure associated with mental distress in the struggle to live.

5. The lipoid of the cortex is stored and lost under conditions entirely different from those which govern the other fats of the body. It does not disappear in extreme bodily emaciation.

6. The cortical lipoid vanishes with great rapidity in all acute febrile infections.

7. There is a tendency for it to appear in excess in chronic renal disease, especially in conjunction with athero-sclerosis.

REFERENCES.

1. Anitschkow, *Ziegler's Beitr. z. path. Anat.*, Jena, 1913, lvi. 379, and 1913, lvii. 201.
2. Arnold, *Virchow's Archiv f. path. Anat. u. Physiol.*, Berlin, 1866, xxxv. 64.
3. Aschoff, *Ziegler's Beitr. z. path. Anat.*, Jena, 1909-10, xlvii. 1.
4. Bernard, *Revue de Méd.*, Paris, 1907, xxvii. 977.
5. Bernard, Bigart, and Labbé, *Compt. rend. de la Soc. de Biol.*, Paris, 1903, lv. 120.
6. Biedl and Wiesel, *Pflüger's Archiv f. d. ges. Physiol.*, Bonn, 1902, xci. 434.
7. Bittorf, *Die Pathologie der Nebennieren*, Jena, 1908.
8. Borberg, *Skand. Archiv f. Physiol.*, Leipzig, 1912, xxvii. 342.
9. Borberg, *ibid.*, 1913, xxviii. 115.
10. Cannon and de la Paz, *Amer. Journ. Physiol.*, 1911, xxviii. 64.
11. Chalatow, *Ziegler's Beitr. z. path. Anat.*, Jena, 1913, lvii. 85.
12. Chauffard, Laroche, and Grigaut, *La Semaine médicale*, Paris, 1911, xxxi. 577.
13. Chauffard, *Compt. rend. de la Soc. de Biol.*, Paris, 1911, lxx. 276 and 855.
14. Chauffard, &c., *ibid.*, 1912, lxxiii. 23.
15. Comessatti, *Archiv f. exp. Path. u. Pharm.*, Leipzig, 1910, lxii. 190.
16. Dreyer, *Amer. Journ. Physiol.*, 1899, ii. 203.
17. Elliott, *Journ. Physiol.*, Camb., 1912, xliv. 376.
18. Elliott, *ibid.*, 1913, xlvi; *Proc. Physiol. Soc.*, 15.
19. Elliott and Armour, *Journ. Path. and Bact.*, Camb., 1911, xv. 481.
20. Elliott and Tuckett, *Journ. Physiol.*, Camb., 1906, xxxiv. 350.
21. Federici, *Lo Sperimentale*, Firenze, 1904, lviii. 419.
22. Folin, Cannon, and Denis, *Journ. Biol. Chem.*, Baltimore, 1913, xiii. 477.
23. Fränkel, *Archiv f. exp. Path. u. Pharm.*, Leipzig, 1909, lx. 395.
24. Gardner and Lander, *Biochemical Journ.*, Liverpool, 1913, vii. 584.
25. Glynn, *Quart. Journ. Med.*, Oxford, 1911-12, v. 157.
26. Goldzieher, *Die Nebennieren*, Wiesbaden, 1911, 21 and 124; and *Wien. klin. Woch.*, 1910, xxiii. 809.
27. Kaiserling and Orgler, *Virchow's Archiv f. path. Anat. u. Physiol.*, Berlin, 1902, clxvii. 296.
28. Klinkert, *Berl. klin. Woch.*, 1913, l. 820.
29. Lapworth, *Journ. Path. and Bact.*, Camb., 1911, xv. 254.
30. Loeschcke, *Münch. med. Woch.*, 1910, lvii. 1. 48.
31. Marchetti, *Virchow's Archiv f. path. Anat. u. Physiol.*, Berlin, 1904, clxxvii. 227.
32. McNee, *Quart. Journ. Med.*, Oxford, 1913-14, 221.
33. Mott and Halliburton, *Archives of Neurol.*, Lond., 1907, iii. 123.

34. Napp, *Virchow's Archiv f. path. Anat. u. Physiol.*, Berlin, 1905, clxxxii. 314.

35. Neusser and Wiesel, *Die Erkrankungen der Nebennieren*, Wien, 1910.

36. Parkinson, *Trans. Path. Soc.*, Lond., 1907, lviii. 187.

37. Philpot, *Quart. Journ. Med.*, Oxford, 1909–10, iii. 34.

38. Picard, *Archiv f. exp. Path. u. Pharm.*, Leipzig, 1913, lxxiv. 450.

39. Reinhardt and Creutzfeldt, *Ziegler's Beitr. z. path. Anat.*, Jena, 1913, lvi. 465.

40. Schmorl and Ingier, *Deutsch. Arch. f. klin. Med.*, 1911, civ. 125.

41. Stewart, *Journ. Exp. Med.*, N. York, 1911, xiv. 377 ; and 1912, xvi. 502 ; also Stewart and Zucker, *ibid.*, 1913, xvii. 152 and 174.

42. Stoerk, *Wien. klin. Woch.*, 1908, xxi. 282.

43. Thomas, *Ziegler's Beitr. z. path. Anat.*, Jena, 1911, l. 283.

44. Tscheboksaroff, *Pflüger's Archiv f. d. ges. Physiol.*, Bonn, 1910–11, cxxxvii. 59.

45. Voegtlin and Macht, *Journ. Amer. Med. Assoc.*, 1913, lxi. 2136.

46. Wacker and Hueck, *Archiv f. exp. Path. u. Pharm.*, Leipzig, 1913, lxxiv. 416.

47. Weltmann, *Ziegler's Beitr. z. path. Anat.*, Jena, 1913, lvi. 278.

48. White, *Proc. Roy. Soc. Med.*, Obstet. Sec., Lond., 1911–12, vii. 247.

49. Wiesel, *Wien. med. Woch.*, 1907, lvii. 674.

DESCRIPTION OF DRAWINGS.

Plate 5, Fig. 1. *Loaded Gland.* The cortical cell columns are laden with scarlet-stained lipoid: doubly refractive crystals are not represented. In this fully loaded state no blue stain is taken by the cell except in the nucleus. The drawing illustrates a chance feature of interest. The adrenal was unusually adherent to the kidney, and a vagrant renal tubule (*r.t.*) lies twisted in the substance of the cortex, which it penetrated nearly as far as the medulla of the gland.

Plate 5, Fig. 2. *Exhausted Gland.* The outer zone of the cortex is figured in the drawing. The cells are for the most part empty of fat. Blood lies in the capillaries (*c.*) between the cortical columns, and also in distended spaces (*s.*) apparently within the columns. The patient died of asphyxia in septic meningitis: fever had exhausted the cortical fat, and the asphyxia caused haemorrhage and congestion. The small blue cells (*b.*) at the periphery are immature cortical cells.

Plate 6, Fig. 3. *Loaded Adenoma in exhausted Gland.* To the right are the scarlet-stained cells of the adenoma (*ad.*), arranged in typical masses within a capillary network. The adenoma is enveloped by a thin shell of dark blue medullary cells (*med.*), which were carried outward as the adenoma spread from its origin in a cortical islet in the medulla. To the left of this are the cortical cell columns (*col.*), slightly distorted by pressure, and exhausted of lipoid except in the extreme peripheral rim. The exhaustion was caused by fever.

Plate 6, Fig. 4. *Cortical Cells in Addison's disease.* Some of the cortical cells are large and laden with lipoid (*l.*); others are strangled by inflammatory round cells and exhausted (*e.*). The giant cell systems and the hyaline fibrosis of the tuberculous inflammation occupy the areas from which the cortical cells have vanished (Case 34).

Plate 7, Fig. 5. *Islet of Adrenal Cortical Cells embedded in Kidney.* The cells are loaded with lipoid and identical in all respects with those of the normal cortex. They grow freely intermingled with those of the renal tubules and without a capsule of demarcation. Cells of a similar character may be recognized in Grawitz's variety of malignant renal tumours. In this case the adrenal gland was closely attached to the kidney and offshoots from its cortex could be traced directly into the heart of the kidney.

Article No. 4.
90'

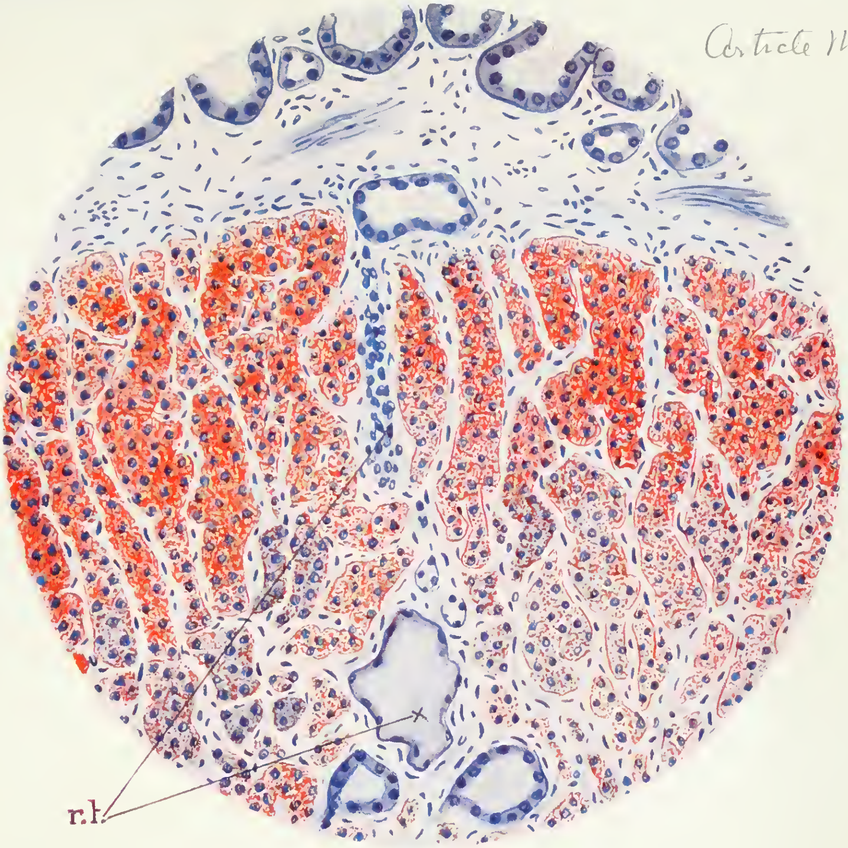
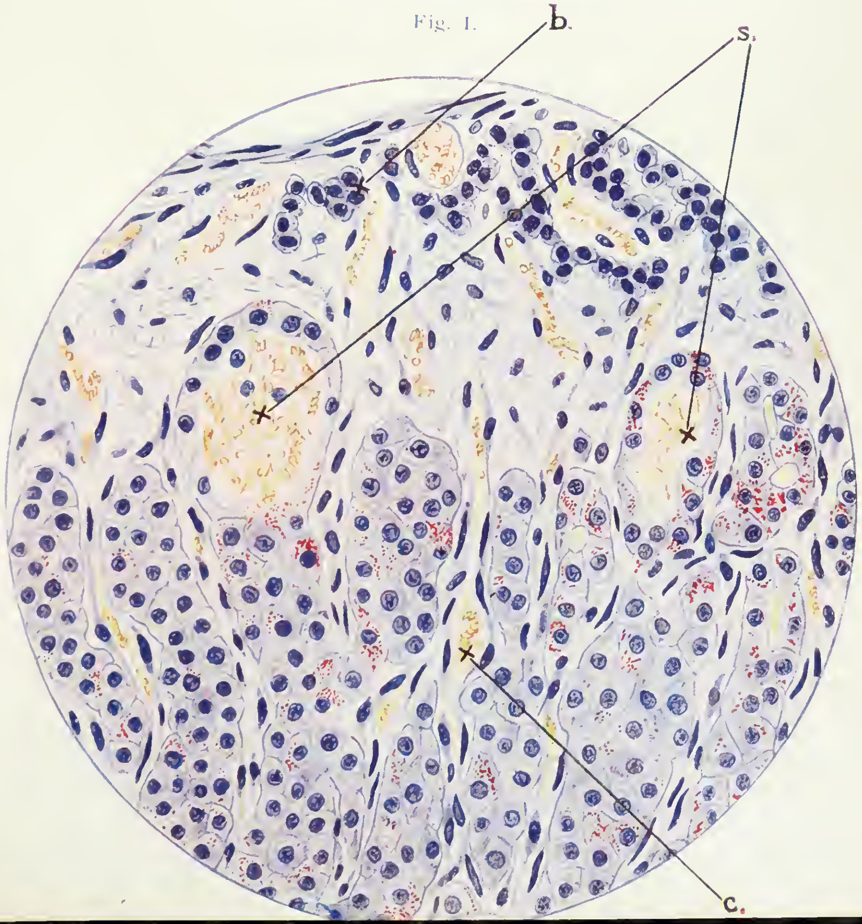


Fig. 1.



Article No. 4
90²

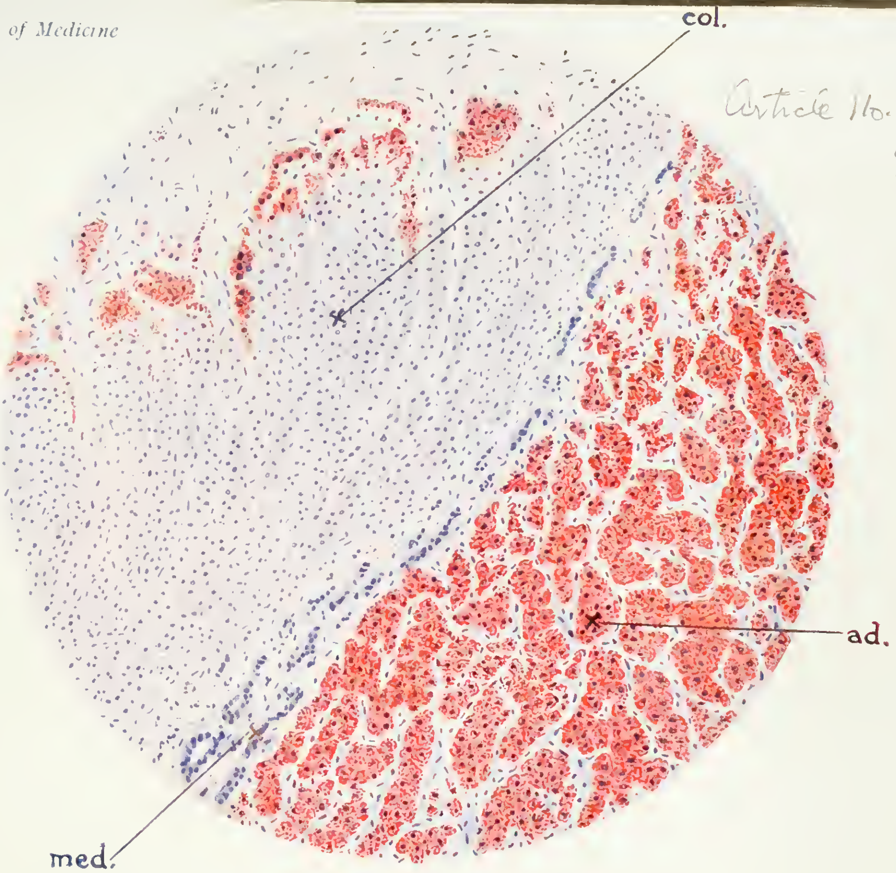
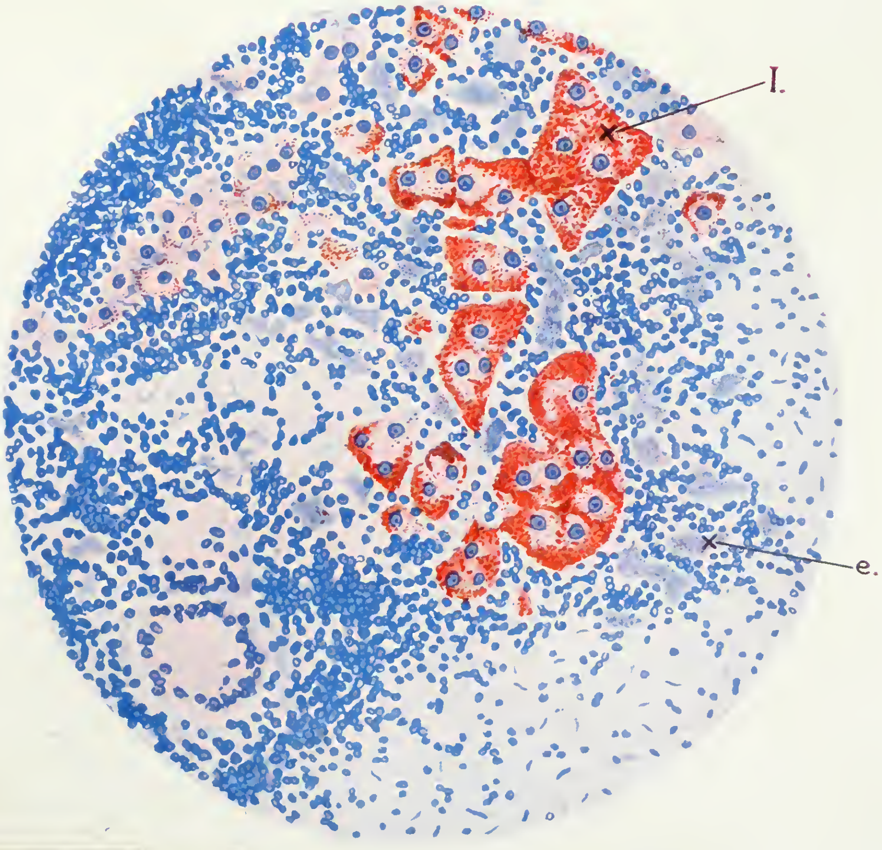


FIG. 3.



Testicle No. 4. 90^{3}.

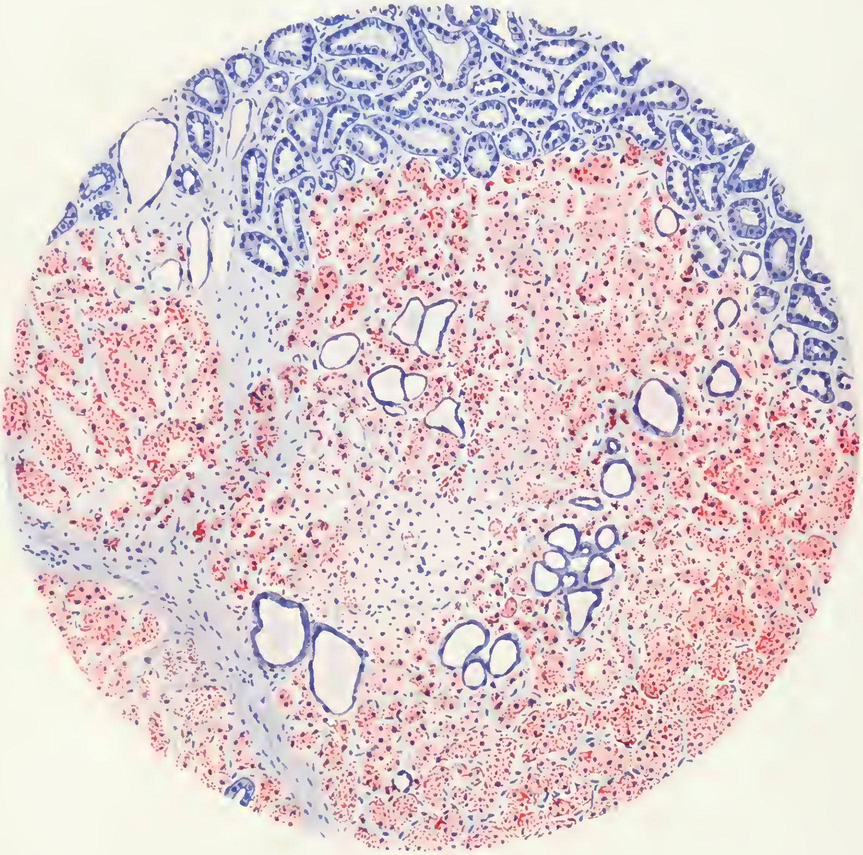


Fig. 5.

[*Reprinted from* "*Heart.*"
Vol. VII, No. 2, July, 1919.]

105

pp 105 – 110.

FURTHER REMARKS ON VENTRICULAR EXTRASYSTOLES AND FIBRILLATION UNDER CHLOROFORM.

By A. GOODMAN LEVY.

(*From the Research Laboratories, University College Hospital Medical School.**)

Since the publication of former papers[1 2 3] on this subject further experiment has made it evident that the term "light chloroform anæsthesia" requires closer definition in its relation to ventricular extrasystoles and fibrillation. The results in the papers referred to were obtained by placing an animal fully, but not deeply, under chloroform, in order to perform the necessary small operative procedures, and then allowing it to revert to a condition of light anæsthesia for purposes of the experiment. On the strength of these experiments it was concluded that the mammalian heart was made irritable by chloroform, that the irritability was raised under light anæsthesia and lowered under deep anæsthesia, and that a group of conditions (or exciting causes) which tend to stimulate the ventricles can make this irritability manifest.

It would appear, however, from the experiments to be now described that the irritability is not readily developed if the animal inhales only a low percentage of chloroform from the beginning, that is to say, if the heart has never been more than very lightly affected. This form of light chloroform anæsthesia may, for the purposes of this paper, be termed "primary" light anæsthesia.

Illustrative Experiment.

Cat. The preliminary operations were performed under the influence of ether, administered by artificial respiration. The vagi were cut in this experiment—rate of heart beat immediately before section 240 per minute and the same immediately after section.

10.41 a.m. A faint corneal reflex. Blood pressure 170 mm.; 0·4 per cent. chloroform vapour substituted for the ether vapour.

---

* A grant in aid of this research was made from the Graham Research Fund, University of London.

10.56 a.m. Active corneal reflex. Blood pressure reduced to 108 mm. and heart beat 210 per minute. 0·064 mgm. adrenalin injected into saphenous vein after 15 minutes inhalation of 0·4 per cent. chloroform. The blood pressure rises to 196 mm. and beat rate to 260 per minute, but the manometer curve shows a continued perfect regularity of the heart beat (Fig. 1). The percentage of chloroform was then gradually increased up to 2 per cent..

11.1 a.m. Chloroform 2 per cent.. No corneal reflex. Blood pressure 60 mm.; heart beat 156. Chloroform gradually reduced to 0·4 per cent..

11.11 a.m. 0·4 per cent.. Blood pressure 108; pulse rate 192.

11.16 a.m. 0·4 per cent.. Fairly active corneal reflex. 0·064 mgm. adrenalin injected. The heart beat assumed the typical character of a multiple tachycardia, terminating in ventricular fibrillation (Fig. 2).

This experiment was repeated under precisely similar conditions with a precisely similar result.

In a third experiment in which 0·5 per cent. chloroform was administered for 15 minutes, and in which the vagi were intact, extrasystoles followed on the first injection of 0·064 mgm. adrenalin, interspersed with regular beats during a period of 45 seconds, but for a few seconds only in a moderately high grade of complexity. The chloroform was then increased to 1·2 per cent., and subsequently reduced to 0·5 per cent., when the injection of the same quantity of adrenalin caused ventricular fibrillation.

On repetition of this last experiment a multiple tachycardia resulted on the first injection followed by momentary fibrillation. Permanent fibrillation resulted from the second injection.

In a fifth experiment (vagi intact), 0·3 per cent. chloroform was first administered for 31 minutes, sufficient ether being continued simultaneously to ensure immobilisation of the animal. Adrenalin was then injected and resulted in an acceleration of the heart beat from 108 to 216 per minute, with the advent of a few extrasystoles only. The chloroform was then increased up to 2 per cent. and again reduced to 0·3 per cent., ether being administered simultaneously as before. The second injection of adrenalin resulted in a multiple tachycardia and fibrillation with subsequent recovery.

Broadly speaking, it may be said that 0·5 per cent. chloroform given for 15 minutes and *never exceeded,* may or may not sensitise the ventricles sufficiently to react intensively to adrenalin. Lower percentages than this do not properly sensitise the ventricles, even if given for longer periods.

The foregoing experiments were suggested by a paper published by E. Nobel and C. J. Rothberger in 1914,[4] detailing electrocardiographic observations on animals under chloroform. The authors state in their introduction that " our researches should not be regarded as a test of Levy's statements as we, for reasons to be presently mentioned, frequently deviated from the methods chosen by him; on the same ground our partly differing results should not be accepted off-hand as a contradiction of Levy's assertions." Nevertheless the deductions drawn from these experiments are sufficiently at variance with the conclusions of Lewis and myself, that they cannot be passed over without comment. To quote the authors further, "Contrary to Levy we have almost entirely worked on tracheotomised animals, and thereby not only obviated the stimulation of the sensory nerves of the upper air passages by the chloroform, but likewise apparently the asphyxia which may be induced partly by the increased secretion of mucous. and partly by the mask covering the head." To this supposed asphyxial element they attach considerable importance, but in this supposition the authors are entirely in error, for not only were my conditions always carefully regulated to avoid it (see page 322 of my article[2]), but likewise a considerable proportion of my experiments were performed under artificial respiration. The reaction shown in Fig. 2 was obtained under artificial respiration, as likewise was the entire series of five experiments described in this paper. In their series of experiments on cats Nobel and Rothberger first performed the necessary operative procedures under ether

Fig. 1.

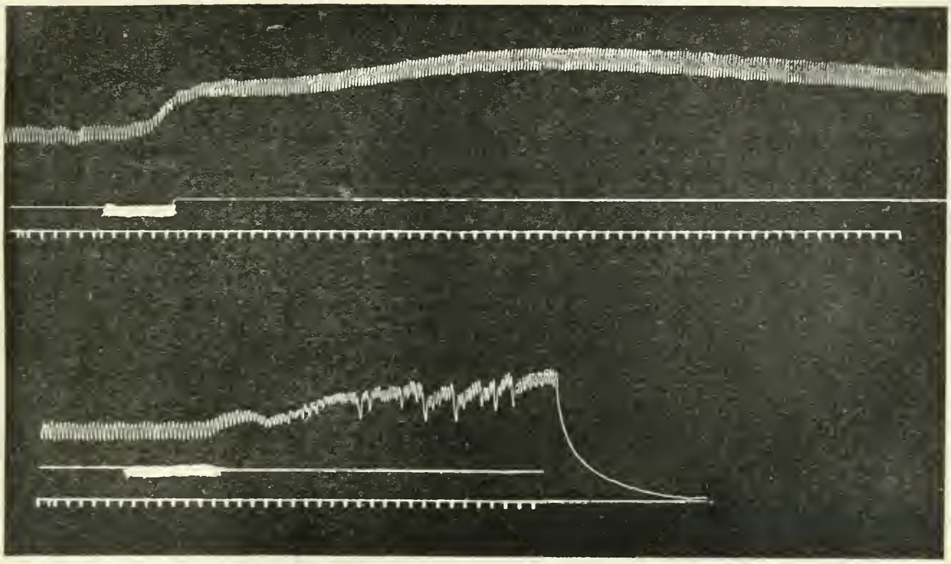


Fig. 2.

Figs. 1 and 2. Two Hürtle curves from the same cat, illustrating the effect of the intravenous injection of 0·064 mgm. of adrenalin (Fig. 1) under primary light chloroform anæsthesia; (Fig. 2) under secondary light chloroform anæsthesia. The signal marks denote the injection. In Fig. 1 the signal line has been adjusted to zero pressure; in Fig. 2, the time line. For full description see text. The time-marker is in seconds.

narcosis; the animals were allowed to come round and then chloroform was administered in a low percentage. That this is an unsatisfactory method of studying ventricular phenomena under light chloroform narcosis has been shown by the experiments I have described above, but these observers fell into a yet more grave error in their technique. The form of inhaler they employed was the same as was used in my experiments; it was supplied at Dr. Nobel's request and the proper method of using it for animals described. Nobel and Rothberger, however, connected the trachea tube directly to the inhaler, allowing expirations to escape by a side tube under water. This apparatus was primarily constructed to work by the suction of human respiration, and the relatively feeble respirations of the cat are quite insufficient to set up a proper draught over the chloroform. I have performed an experiment with the apparatus used in this manner, and I found that after a fifteen-minutes administration of a nominal 0·5 per cent. chloroform vapour that the blood pressure had not dropped at all (*c.f.* experiment on page 105), and that the injection of adrenalin only set up a few irregularities in the tracing such as are frequently observed under ether narcosis alone. Further I found that even with a nominal 3 per cent. vapour it was impossible to maintain efficient narcosis. I conclude that with a setting of the index hand at 0·5 the animal only inhales the barest trace of chloroform vapour, if, indeed, any at all.

The whole of their series of experiments upon cats are adversely affected for this reason. It is impossible to decide the actual degree of chloroform anæsthesia under which the observations were made, and in certain cases there is little doubt the animals inhaled practically no vapour at all.

A further series of experiments was performed upon dogs. In these animals the respirations are more powerful and no doubt the vapour inhaled by natural respiration through a tracheal tube may have some approximation to that indicated on the index scale, and in fact the results obtained were more significant than in the case of the cats; complex tachycardias were obtained, and in one instance permanent ventricular fibrillation resulted. There are, however, two further sources of error in these experiments which invalidate the observations. The "light anæsthesia" is again that of primary light anæsthesia, which is a different condition to that under which my experiments were performed. Secondly, the dogs were morphinised, in order, as the authors state, to increase vagal activity. They were heavily morphinised, 0·25 gramme being injected subcutaneously, and apparently to a degree sufficient to allow of the preliminary operations being performed without further narcosis. Under such circumstances I regard these experiments as being of an entirely different order to my own, which were performed under the simplest possible conditions. The effect of increased vagal tone is to diminish the liability to ventricular irregularities, and distinctly affects the incidence of ventricular fibrillation adversely (see page 357 of my article[2], and page 325 of another article[3]). Whereas Nobel and Rothberger only obtained permanent ventricular fibrillation in a single dog under these conditions, they found that by following up the adrenalin injection by 1·5 mgms. of atropin the ventricular tachycardia frequently terminated in fibrillation, thereby, I think, fairly demonstrating the error of their method, for the atropin may be regarded as neutralising the increased vagal tone induced by the morphia. In this connection it is noteworthy that atropin alone is capable of producing, in the lightly chloroformed animal, a multiple tachycardia generally terminating in fibrillation, as pointed out on page 317 of my paper,[3] a fact of which the authors appear to be unaware.

In their "discussion" the authors make the following statements, which may certainly be taken to contradict my results :—

(1) "Whilst Levy always found arrhythmia to occur in normally breathing cats under light anæsthesia, we have always missed this in tracheotomised dogs and cats." This is not a correct statement of my conclusions. My statements in this regard are quite clear, and show that the onset of the arrhythmia is conditioned by the decrease or removal of the chloroform. "It is not my view that chloroform in a particular degree of concentration can initiate a specific form of irregularity, but the onset of irregularities is, I believe, conditioned by the *change of cardiac state* involved in the progress from deep to light anæsthesia." Again,[2] "the heart may be maintained beating at a perfectly regular rate even when lightly anæsthetised so long as the anæsthesia is a level and unchanging one, and no other disturbance is at work, such as may arise from sensory stimulations (see page 365)." The conditions of Nobel and Rothberger were certainly prejudicial to eliciting these irregularities, that is to say, of a primary light unchanging chloroform anæsthesia, of probably infinitesimal degree in cats, and complicated with morphine narcosis in dogs.

(2) "Stimulation of sensory nerves has further in Levy's researches led to arrhythmia under light narcosis, whereas in our researches on tracheotomised animals it was ineffective." Similar objections hold good in this instance. Nobel and Rothberger performed three experiments with negative results, whereas I have found reflex irregularities to occur with great constancy in over fifty observations on cats under secondary light chloroform anæsthesia. I have recorded positive results also in animals under artificial respiration, so that it is evident that the negative results were not conditioned by freedom of pulmonary ventilation, but solely by the adverse influences referred to.

(3) "The injection (of adrenalin) under light narcosis almost always led to a disturbance of rhythm ; this was in our experiments altogether less intensive than in Levy's, who says that 0·064 mgm. adrenalin almost certainly led to ventricular fibrillation under light narcosis." The experiments described earlier in this paper demonstrate not only how this divergence may arise, but likewise that intensive results are obtained perfectly readily even when the lungs are freely ventilated. A statement is likewise made several times in the body of the paper that adrenalin caused

a higher degree of irregularity under deeper narcosis than under light; this is no doubt partially correct under the conditions of primary light anæsthesia employed, but entirely contrary to what is observed following fully established anæsthesia. At the same time it is doubtful whether what Nobel and Rothberger describe as deep anæsthesia was really such, for they were guided by the readings of the index scale of the inhaler, which, as employed by them, could not work accurately.

The authors further do not appear to agree that chloroform makes the ventricles irritable in the sense employed by me, and they regard the adrenaline effect as due to a combined vagus and accelerator action; in fact, they are inclined to bring the chloroform phenomena into relation with an action upon the hypothetical tertiary "Reizbildungszentren" which are frequently referred to in Rothberger and Winterberg's writings. It is, however, certain that the adrenalin reaction occurs independently of all extraneous nervous action; this point is definitely settled by experiments already recorded by me (see page 330 of my article[2]), but to which Nobel and Rothberger make no reference.

It is noteworthy that in certain experiments in which our experimental methods were identical, there is no divergence of opinion. Thus Nobel and Rothberger agree that adrenalin does not disturb the normal sequence of the heart beat in an animal not under an anæsthetic, and it is interesting to have an electrocardiographic confirmation of this fact. They also demonstrate the nature of the minor ventricular irregularities caused by adrenalin in the etherised animal, to which I have referred in my writings. However, to sum up, Nobel and Rothberger's experiments are generally vitiated by a method of primary light chloroform anæsthesia which fails to sensitise the ventricles, by a faulty method of administering percentage vapours, and by the preliminary use of morphia. Their peculiar results are due to these conditions, and not to the fact, as they presume, that their animals were tracheotomised.

REFERENCES.

[1] Levy and Lewis. Heart, 1911-12, iii, 99.

[2] Levy. Heart, 1912-13, iv, 319.

[3] Levy. Heart, 1913-14, v, 299.

[4] Nobel and Rothberger. "Uber die wirkung von Adrenalin und Atropin bei leichter Chloroformnarkose." Zeitschr. f. d. ges. exper. Med., 1914, iii, 151.

Hi. 6

11

VI.—*The Spread of the Excitatory Process in the Vertebrate Heart.* Parts I–V.

By Thomas Lewis.*

Communicated by Prof. E. H. Starling, *F.R.S.*

(From the Cardiographic Department, Graham Research Laboratories, University College Hospital Medical School.)

(Parts I–III and Part V received June 21, Part IV received August 30,—Read November 11, 1915.)

Part I.—*The Toad's Ventricle.*

[Plates 15 and 16.]

Introductory.

If non-polarisable electrodes are placed upon the body of the toad, the one contact lying on the muscle near the root of the neck in front and on the right side, while the other contact rests on the muscle in the centre of the abdominal wall, and these contacts are united to the string galvanometer in such a way that relative negativity of the cephalic contact yields in our photograph an upward excursion; then the form of the curve which is written at each cycle of the heart may be exemplified by the upper curve of fig. 1 (next page).

This curve is representative, and is a record of events which are almost uniform when the heart is beating normally and its rate is about 25 beats to the minute. It consists of an upward, rounded, and sometimes subdivided deflection, P; this deflection is followed by a period of rest, during which the string lies on the zero line or near it. Ventricular activity is first signalled by the summit R, and this elevation invariably begins in a gradual fashion, its steepness increasing as it proceeds. The string, after a variable interval of from 0·0400 to 0·0900 second, falls back towards the base line. It may or may not attain that line, but it usually does, and, travelling somewhat beyond, produces a diminutive deflection S; this ends the first part of the ventricular complex. Then follows a second period of rest, which is finally disturbed by a slow and upward movement of the string in the inscription of the deflection T. In many cases a notch, B, is found in the interval between S and T.† (The little deflection B has usually a downward direction, and is the result of activity in the *truncus arteriosus.*)

Such is the customary and almost constant form of the axial electrocardiogram in an extracardial lead, and, by common consent, P represents activity of the auricle,

---

* Working under the Medical Research Committee. A preliminary report of the observations appeared in the 'Proceedings of the Physiological Society,' June 5, 1915.

† The P–R interval and the R–B interval in this curve are of more than average duration.

[Published, March 3, 1916.

while the remaining deflections represent the activities of the ventricle. Of these deflections, *R* and *S*[*] may be regarded as expressing the spread of the excitation wave in the ventricle; the horizontal line between *S* and *T* may be regarded as expressing equal activity of all parts of the ventricle, and *T* may be regarded as expressing the decline of the excitatory stage. But until recently these axial extracardial curves have received little attention. In earlier works the contacts were placed upon the muscle of the heart. The curves which Sanderson and Page[†] took with the capillary electrometer were from contacts placed upon the base and apex of the ventricle. These writers state[‡] that when such a lead is adopted two deflections are recorded, the first of which indicates basal negativity (an upstroke), and the second of which indicates apical negativity (a slow downstroke). The two deflections of opposite sign described by Sanderson and Page have been recorded by many experimenters, and out of this observation the impression has grown that the excitation wave proceeds as a simple muscle wave from base to apex of the heart. But recently it has been ascertained by Samojloff[§] and Gotch[||] that in certain base-apex leads the second slow deflection has the same direction as the primary deflection, and Gotch, who appears to have been prompted in the first place by this fact, ventured the hypothesis that the excitation wave travels from base to apex and ultimately returns to the base. He found confirmation of this view in an inconspicuous movement of the recorder in a downward direction, at a time corresponding somewhat closely to *S* in fig. 1. Briefly, the base-apex curve was said to be triphasic, comprising (1) a base-negative effect, (2) an apex-negative effect, and (3) a base-negative effect.

It is requisite, before proceeding further, to examine this direct method of leading more closely. In fig. 1 are two curves, the upper of which has been described.

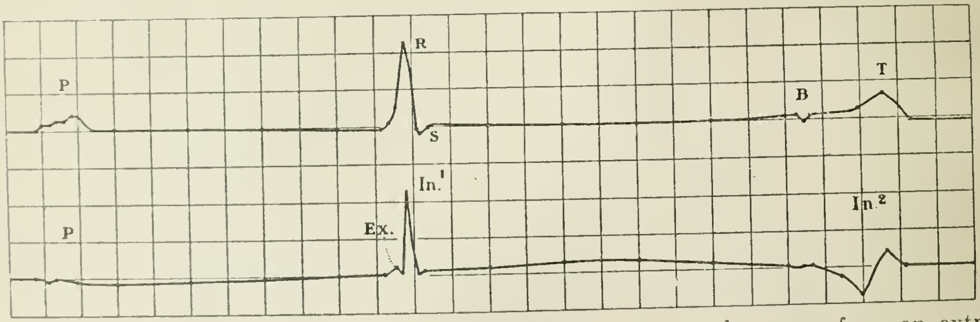


Fig. 1.—A chart constructed from two simultaneously written curves—the upper from an extracardial base-apex lead, the lower from a direct base-apex lead. The abscissæ represent tenths of seconds; the ordinates of the upper curve are on the scale of 2 millivolts to the centimetre (two scale divisions), and of the lower curve 2 millivolts to the *millimetre*.

---

[*] The amphibian electrocardiogram shows no *Q*.

[†] 'Journ. of Physiol.,' vol. 4, p. 327 (1883–84).

[‡] 'Roy. Soc. Proc.,' vol. 27, p. 410 (1878).

[§] 'Archiv f. d. ges. Physiol.,' vol. 155, p. 471 (1914).

[||] 'Roy. Soc. Proc.,' vol. 79, p. 323 (1907).

Below it is a simultaneously written curve, inscribed from a base-apex lead such as SANDERSON and PAGE and GOTCH employed. Upon cursory inspection it presents similar features to the upper curve, and the deflections In^1 and In^2 are the deflections recorded by these workers and recognised by physiologists ever since their papers were published.

But when we analyse the two curves more carefully we find they differ in certain very essential particulars. In the first place, the sensitivity of the recording fibre of the upper curve was ten times as great as that of the lower curve, and this greater sensitivity in the case of the extracardial lead was not necessitated by the greater resistance in circuit, for by the method of standardisation this extra resistance is taken into account.*

We may say, therefore, that the potential difference developed in the lower curve at the time R was written was ten times as great as in the upper curve.

But the curves differ in another very essential respect. The upstrokes R and In^1 are not simultaneous; R has a start of some 0·0450 second.† The first two-thirds of the upstroke R are written before In^1 starts, and the latter has a far steeper rise than the former. Consequently, we may affirm that these upstrokes are not due to the same events. Moreover, the deflection In^1 is preceded by a summit Ex, which has no counterpart in the upper curve.‡

These and other observable differences are due to the change of lead, and may be explained in the following manner. In the extracardial lead the whole heart lies between the electrodes; the relation of the upper contact is very similar to all parts of the heart's base, and the relation of the lower contact is very similar to each part of the apex. The curve represents as fully as any single curve can the sum total of the heart's activities and the individual activities proportionately. But where the contacts are placed on the muscle at base and apex, the points chosen for contact are favoured points, and our curve then consists of (1) a general expression of the activities, and (2) a special expression of the activities of muscle beneath the contacts. In a paper written with MEAKINS and WHITE§ I have shown that the curves of direct leads comprise effects of these two kinds, which are termed *extrinsic* and *intrinsic*, respectively. The intrinsic effects are those signalling events which occur in the immediate neighbourhood of the contacts; the extrinsic effects at some distance from them. In all direct leads from the heart, whatsoever the method employed, these two sets of effects are intermixed and are often very difficult to disentangle. Until complete separation is possible, and until the extrinsic effects are more fully understood than they are at present, we cannot be too circumspect in interpreting curves taken by leads direct from the heart muscle.

---

* With circuit resistances of from 20,000 to 40,000 ohms the instrument may be regarded as a potentiometer; standardisation is obtained by throwing a known E.M.F. into circuit and obtaining for that E.M.F. a given excursion.

† Measurements made upon the original negative by comparator, as in previous papers.

‡ In curves taken with the capillary electrometer these small initial phases seem to have been lost.

§ 'Phil. Trans.,' B, vol. 205, p. 375 (1915).

Adapting our terminology to fig. 1, we may describe the whole upper curve as uniformly extrinsic in origin. In respect of the lower curve, *P* the auricular deflection is evidently extrinsic. The initial ventricular deflection *Ex* is also extrinsic. I have been at pains to show, by observations upon the dog's auricle and ventricle, that the first intrinsic deflection signals the arrival of the excitation wave immediately beneath one contact, and it may be recognised by its direction and by its steepness. Thus, when the wave of activity is prevented from reaching the contact, this deflection no longer appears in the curve; the later deflections, represented in our present curve by In^2, are abolished at the same time. These deflections are due to some local change, while *P* and *Ex*, unaffected by the same procedure, are due to events occurring at a distance.

How much of the initial movement In^1 is due to local change under the basal contact, and how much to local change under the apical contact, and, further, to what extent it is complicated by hidden extrinsic effects, become matters for enquiry. These questions emphasise the precise difficulties which confront the interpretation of each direct lead from two contacts placed upon the ventricle. Move the extra-cardial contacts to one or other side over a considerable range and there is little perceptible change in the curve; move one of the direct contacts ever so little and change of form, often profound, is the almost invariable result. The lower curve of our figure is not characteristic of a direct base-apex lead in the toad; no such curve is characteristic; curves of many shapes, and separate curves with the chief deflections in opposite directions, may be obtained in one and the same animal. So changeable is the form, and so varied and so many-phased, that deductions from such leads are often precarious and may be most misleading. The present communication deals only with the preliminary phases of the ventricular curve, represented in fig. 1 by *R* and *S*, to which attention may now be confined. The animal which gave the base-apex curve of fig. 1 also gave, by a slight movement of the basal contact, the base-apex lead of fig. 2, *I*. Here the initial phases are alone plotted, and on a different scale, but the wide departure from the initial phases of fig. 1 (*Ex*, In^1) is at once manifest. Some insight into the composite character of this curve may be gained by studying the curves from the companion leads, illustrated below the chart. The three curves of the chart are plotted on a uniform scale and with the corresponding time phases vertically above each other. The three contacts used were (1) base, (2) apex, (3) body wall, and the leads were arranged around the sides of a triangle (*I*, base to apex; *II*, base to body wall; and *III*, apex to body wall). Now Leads *II* and *III* represent our accustomed method of leading direct from the heart; one contact lying on the muscle, the other on an indifferent point; and such curves resemble each other in one respect; after the inscription of certain initial phases, usually downward in direction and relatively slow in movement, they are broken by a steep rise, which signals relative negativity of the corresponding heart contacts. As we find that this rise corresponds to the arrival of the excitatory process beneath the contact, the advantage of placing a single contact upon the ventricle is clear; we avoid the clashing of local events under two contacts of a pair when each contact lies on the muscle. By our method, the direction which the deflection, signalling the arrival of the excitation process, will take is known beforehand; such deflections are steep upstrokes. Leading thus from base and apex, each to body wall, the corresponding intrinsic deflections are sharply defined, and we observe in the present instance that the apex becomes active before the base (actually by 0·0070 second). We may now read into the *base-apex* lead the same event, the first sharp *downstroke* of this curve is evidently due to relative negativity of the apical, as opposed to the basal, contact, for it occurs at almost the same instant as the upstroke of the apex to body wall lead. But if we read the first curve upon the accustomed plan we must acknowledge that the first event in the base-apex lead is an indication of relative negativity of the base, for a rise precedes the fall. The initial phases of this curve are clearly of the same origin as the initial downstrokes of Leads *II* and *III*; they are extrinsic effects. *Thus, we are not justified in concluding from the direction of the first deflection in the base-apex lead, that base or apex first becomes active.* Neither are we justified in using the first prominent deflection of such a lead as an indication, for it not infrequently happens that the chief extrinsic deflection is as prominent as the intrinsic deflection. We *are* justified in using the most prominent upward deflection of leads such as *II* and *III* as indications, providing certain precautions are observed in doubtful instances. These remarks lead up to my final observations. My view is distinctly that the base-apex lead or any lead comprising two contacts on the ventricular surface must for the moment be abandoned in favour of other methods.

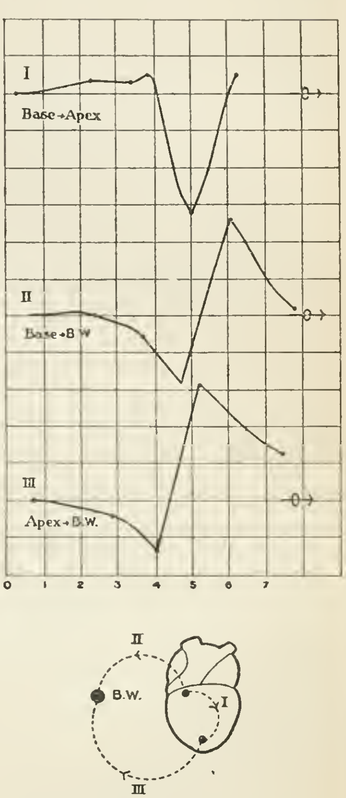


Fig. 2.—A chart of three curves taken from the same animal as fig. 1, but representing initial phases of ventricular curves only. The abscissæ represent hundredths of a second; the ordinates 1 millivolt to the millimetre. The curves are acceptable because completing a triangle they obey the formula *I* + *III* = *II* (see p. 234). Below is an outline of the heart and corresponding leads to scale. *B.W.* = Body wall.

Clement,* who hitherto has alone attempted to map out the course of the excitation wave in the frog's ventricle, has adopted a special method of leading. He uses a thread bent at an angle, the ends of which are in circuit with the recording instrument; he applies the angle of the bent thread to the surface of the heart, and thus obtains an ingenious form of electrode of which the two contacts may be regarded as in very close approximation. Nevertheless he does not avoid the pitfalls of such direct double contact leads; his curves clearly show the presence of extrinsic effects*; many are diphasic, consisting of a preliminary extrinsic and a final intrinsic deflection of almost equal extent; to my mind his deductions, both from the direction of the prominent deflection and from the time at which it has its beginning, are open to very serious objections; and the discrepancies between his conclusions and those which I now propose to narrate are to be explained by the difference in our method of observation.

---

* 'Zeitschrift f. Biol.,' vol. 58, p. 110 (1912).

Surface Distribution of the Excitation Process.

The observations upon which this report is based were made on large female toads of the species *Bufo vulgaris major* in the months of March and April. The heart rates in different experiments varied from 20 to 29 per minute. The toads were pithed, brain and cord being completely destroyed; the vagi were left intact.

Readings of the commencement of the excitatory process were taken by the method already described by LEWIS and ROTHSCHILD,† the sole difference in procedure being a reduction in the size of electrodes. For direct contacts I have used small bore glass tubing filled with kaolin, and an upper layer of copper sulphate; a cotton thread daubed with moist kaolin and freshened from time to time formed the actual contact; this was paired with a contact on the body wall.

The hearts have been drawn to scale *in situ*, and the outlines of the figures are reproduced at natural size.

In all illustrated experiments the curve of Lead *II* has been used as a standard of measurement. I do not use the upstroke of *R* for this purpose, but its summit, for the commencement of the curve is slow and cannot be estimated with sufficient constancy. The readings as given in this paper are all related to the commencement of *R*, however; the interval between upstroke and summit being measured in a number of plates, and the average interval being allowed in the calculation of final readings (Plate 15, fig. 7).

The Ventral Surface.—Readings from the ventral surface of the heart are illustrated by figs. 3, 4, and 5. In a given animal the total variation in the intervals is from 0·0150 to 0·0350 second. The excitation wave does not appear simultaneously over the ventral surface, as CLEMENT has stated. The order of the readings is variable: almost every rule which may be formulated is broken by frequent exceptions.

---

* I would point out that CLEMENT'S control of his method is an experiment upon the sartorius muscle, where conduction is upon a simple plan and where the confusion of the extrinsic deflection is not encountered.

† 'Phil. Trans.,' B, vol. 206, p. 181 (1915).

In three instances all basal readings, *i.e.*, those taken along the *A–V* groove, are higher than the extreme apical reading (figs. 3 and 5, *E* and *F*), by values of 0·0100–0·0300 second or more; in two other instances one or more of the basal readings is somewhat higher than the extreme apical reading (fig. 5, *D* and *G*). In three instances (fig. 4) all or most of the basal readings are lower than the apical reading.

The belief that the base of the heart is activated before the apex is therefore without foundation for the majority of toads; the reverse is usually the case. Neither can GOTCH's conclusion that the wave starts from the base, travelling to the apex and returning to the base, be entertained. It is the rule that some point in the central region always has precedence to base and apex. The earliest region of the ventral surface to become active is the central zone, sometimes actually the centre point (figs. 3 and 5, *E* and *F*) or a point very close to it; but in three instances the earliest point has been towards the left or right margin of the central zone. In only one instance has the earliest point lain outside the central

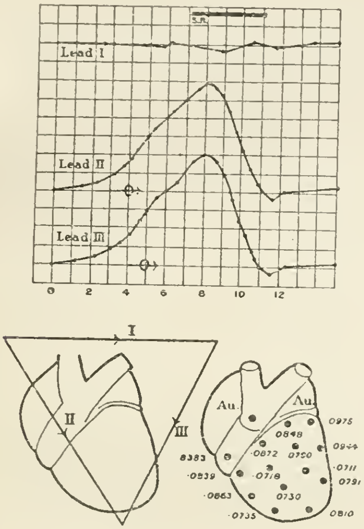


FIG. 3.

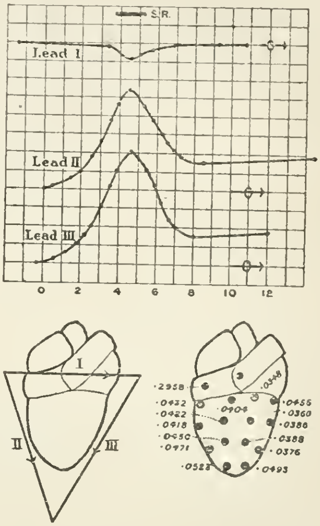


FIG. 4.

FIG. 3.—A chart showing the initial phases of the electrocardiogram in extracardial leads *I, II,* and *III* of Toad *B*. Ordinates, 1 cm. = 1 millivolt; abscissæ, 1 cm. = 0·0400 second. Also two natural size outlines of the corresponding heart to scale. The surface readings of the right-hand figure are accurately related to the commencement of *R* as it is charted. The readings of the deflections in the chart and the surface readings of the diagram may be compared therefore. The left-hand figure shows the relation of the three lead contacts to the heart. The charts of this and the succeeding figure have been re-drawn and are not precisely accurate.

FIG. 4.—A similar chart and outlines of the heart of Toad *C*. The scale of ordinates in this figure is such that 3 cm. = 1 millivolt; abscissæ as in previous figures. The surface readings of the right-hand figure are accurately related to the commencement of *R* as it is charted. The readings of the deflections in the chart and the surface readings of the diagram may be compared therefore.

zone (fig. 4), and in this instance it preceded the earliest point in the central zone by 0·0012 second only.

The left margin of the heart is usually activated either a little before the right margin, or the readings on the two margins are similar.

The Lateral and Posterior Surfaces.—In four animals (fig. 5, *D*, *E*, *F*, *G*), after having taken a series of readings from the ventral surface, the heart has been rotated so as to lie on its side and a series of additional readings has been taken, overlapping the fields of observation. The readings are of much the same order in magnitude as over the ventral surface and show similar variations. Here again the lowest reading is often discovered in the central zone (exceptions will be found in Toads *E* and *G*). In all instances (*D*, *E*, *F* and *G*) the base is as late or later than the apex.

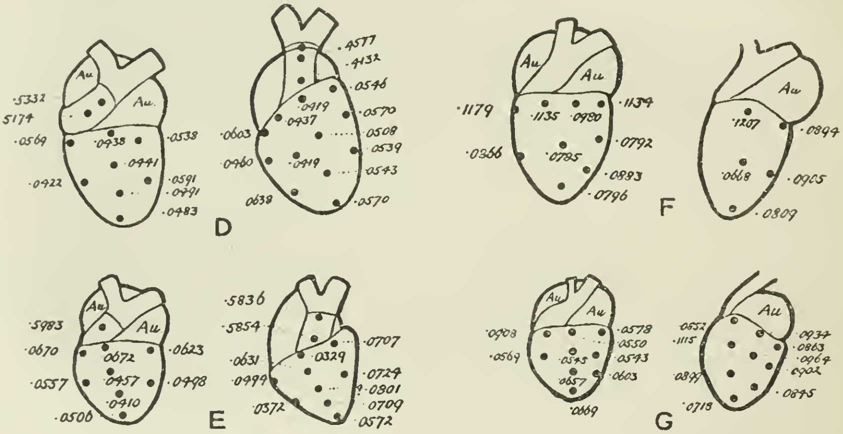


Fig. 5.—Natural size. Outline diagrams to scale of the hearts of Toads *D*, *E*, *F*, and *G*, showing a number of surface readings, expressed relative to the commencement of *R*. In this figure, also, the readings are related to the commencement of *R*; the intervals between the commencements and summits of *R* (the actual standard in measuring) will be found in Table I.

The left-hand outlines of the four paired figures are of the ventral surface of the heart in each instance; of the right-hand outlines those of *D* and *E* are of the right surface, those of *F* and *G* are of the left surface of the heart.

A number of readings from the *truncus arteriosus* are given in the illustrations. This tube is muscular in its first portion, and the limits of the musculature are readily to be recognised in the exposed heart. Contacts placed on the muscle give very late readings. As a general rule, the excitation wave reaches the *truncus arteriosus* a half second after the whole surface of the ventricle is involved. The interval is variable, extending from 0·25 to 1·0 second. The surface change to the high reading is abrupt at the line of junction with ventricle, and there appears to be a natural blocking point between ventricle and truncus. That the excitation wave travels up the truncus may be shown by obtaining readings from the upper and lower ends of the muscular tube. If such readings are taken by separate comparison with the standard curve (as in the figures) the reading from the lower reaches of the conus is usually the lesser value; but it may be of equal value with the reading from the higher reaches of the truncus (as in fig. 5, *E*). The method is insufficient as the ventricle-truncus interval is great, and subject to correspondingly large fluctuations (compare ventral and side readings in fig. 5, *D*). The order of excitation is conclusively shown to be in the direction stated by simultaneous curves taken from two truncus contacts; in these observations the progression is invariably in the cephalic direction (see Table III).

The *truncus* activity is often signalled in the axial electrocardiogram (see fig. 1, and Plate 15, fig. 8), being represented by a shallow dip which falls between *R* and *T*. A lead from the truncus yields two intrinsic phases (Plate 15, fig. 8), *B* the intrinsic deflection proper (see Plate 16, fig. 10), and *BT* the final effect, which is of a similar nature to *T* in the axial curve. It also yields large extrinsic effects from auricle and ventricle (fig. 8, *R* and *T* of lower curve). If the contact is moved off the muscle and on to the artery. the remaining conditions being constant, the intrinsic effects are lost. while extrinsic effects remain (Plate 16, fig. 9).

The Relation of Surface Readings to the Axial Electrocardiogram in Extracardial Leads.

This may be studied in figs. 3 and 4 and by a comparison of the remaining figures with Table I. In figs. 3 and 4, which may be taken as fair illustrations, the initial phases of the electrocardiogram in Leads *I*, *II* and *III* are charted relative to the commencement of *R*. Below the chart the 1/100 second time lines are enumerated; the commencement of *R* stands on the 0 line. All the surface readings fall within the space bounded by the thick line *S R* on the charts. The ventral surface begins to be activated during the time phase which corresponds to the last third of the upstroke *R*. and other points are activated while the summit and first half of the downstroke is written. As the initial phases of the electrocardiogram (*R* and *S*) are supposed to correspond to the activation of the muscle, we enquire why the surface readings do not cover the same time period as the complete initial deflections of the axial curve.

TABLE I.

Toad.	Average heart rate.	R.*	Summit of *R*.	Descent of *R*.	Bottom of *S*.	Return of *S*.	Latest intrinsic reading.
A	37	0·00	0·0836	0·1030	0·1100	None	—
B	24	0·00	0·0828	0·1100	0·1158	0·1220	0·0975
C	26	0·00	0·0454	0·0800	None	—	0·0523
D	29	0·00	0·0618	0·0889	0·0917	0·0990	0·0638
E	27	0·00	0·0429	0·0531	0·0668	0·0736	0·0724
F	27	0·00	0·0725	0·0913	0·1005	0·1243	0·1207
G	26	0·00	0·0688	0·0914	0·1018	0·1408	0·0964
H	25	0·00	0·0885	0·1271	None	—	0·0776
J	24	0·00	0·0682	0·0997	None	—	0·0829
K	20	0·00	0·0505	0·0676	None	—	0·0495

* All readings of initial phases apply to Lead *II* only, and are related to *R*.

The hiatus which coincides with the first two-thirds of *R*'s upstroke we may neglect for the moment. The shorter hiatus, corresponding to the end of the downstroke of *R* and to *S* is readily explained. The readings on the dorsal surface of the heart are in general a little higher in value; this difference may be in small measure responsible. It may be also that the surface of the ventricle which curves into and lies buried in the *A–V* groove is activated later; that such is the case would be anticipated from the usual order of readings: in brief, the latest surface readings are probably not obtained, as the whole muscle is inaccessible. But a certain and chief cause of the hiatus is that our readings are taken from the beginnings of intrinsic deflections, or of a time instant when the surface potential is beginning to develop. How long it continues to develop is uncertain, but, judging from the time taken for intrinsic deflections to reach their full amplitude, time intervals of 0·0100–0·0150 second must be allowed. If we add such time intervals to the readings of the latest intrinsic deflections in the last column of Table I, we shall find close correspondence between the figures at which we arrive and the end of the initial deflections in the axial lead, and so may draw the general conclusion that the *surface excitation is coincident with the initial phases of the axial electrocardiogram in extracardial leads from a point well up the upstroke of R to the termination of these phases.*

Further Observations.

When we consider the surface readings we may conclude from these alone that the excitatory process does not travel as a simple surface wave. For, if that were so, not only would the readings be arranged in orderly succession, but the simultaneous appearance of the wave at points far removed from each other would not be discovered, unless, indeed, spread occurred from a central surface point. But such readings as we possess are not compatible with any such simple mode of surface spread.

To test this question more fully I have made a number of observations upon surface conduction rate.

I have used two direct contacts, *p* and *d*, each paired with an indifferent contact on the chest wall, just as Rothschild and I proceeded in investigating the dog's heart. Stimulation* is in line with *p* and *d*, the heart contacts of the proximal and distal leads. The interval between the two intrinsic deflections written in the simultaneous curves is estimated and the distance between *p* and *d* is measured. My results are given in Table II, the arrows in the last column indicating the direction relative to the ventricle as a whole in which the excitation wave travelled past the contacts in different experiments. With a solitary exception the calculated rates of transmission are sufficiently uniform, yielding an average of 76 mm. per second.

We may compare this rate of conduction of artificial waves propagated over the ventricle with the natural rate of conduction over the *truncus arteriosus* (Table III).

---

* In the estimation of rate, two or more of the first four beats of a succession of responses were alone utilised.

Table II.—Rates of Conduction.

Toad.	Interval.	Distance.	Rate.	Average rate.	Direction of conduction.
	sec.	mm.	mm. per sec.	mm. per sec.	
F.	0·0984*	4	41	42	↖
F.	0·1171	5	43		↗
G.	0·0491	3·2	65	89	↑
G.	0·0252	2·8	111		↖
G.	0·0558	5	90		↗
H.	0·0704	5	71	65	↗
H.	0·0681	4	59		↖
H.	0·0187	6	311†	—	→
J.	0·0507	4·2	83	88	↖
J.	0·0610	4·8	79		↗
J.	0·0417	4·2	101		←
K.	0·0408	5·2	127	96	↗
K.	0·0655	4·3	66		→

Average rate of conduction, 76 mm. per sec.

* Each figure is an average of two readings.

† Excluded from averages.

Note.—It is to be regretted that the temperature of these animals was not taken; the observations were undertaken during mild weather, and the laboratory temperature was usually about 16–18° C.

Table III.—Natural Conduction Rates in *Truncus arteriosus*.

Toad.	Interval.	Distance.	Rate.
	sec.	mm.	mm. per sec
D	0·0173*	2	116
D	0·0253	3	119
J	0·0152	2·8	184
K	0·0240	2·4	100

Average rate of conduction, 130 mm. per sec.

* Each figure is an average of two or more readings.

These estimated rates are higher, averaging 130 mm. per second. An over-estimating of rate is to be anticipated in the *truncus*, for, utilising the natural excitation wave, its precise line of travel is unknown, and any deviation of the line of contacts from the line of travel will of necessity yield an error in this direction.

We may take 100 mm. per second as a safe approximation of the rate of travel in ventricular muscle.*

On this basis, the basis of calculated rate of conduction in the animals concerned, a simple base-apex or apex-base spread in a ventricle having a length of 13 mm. (fig. 3) would have a duration of 0·13 second, over a ventricle of 17 mm. (Toad *A*) of 0·17 second. The complete spread actually occupied 0·0233 and 0·0369 second respectively in these animals. The actual rate of spread is five times greater than can be accounted for in assuming spread from base or apex, and two and a half times greater than can be accounted for in assuming spread from a central focus.

The relative rapidity of spread and the general order of surface readings led me to anticipate a similar form of distribution to that discovered in the dog's heart, namely, spread from within outwards. And the conclusion that such is indeed the mode of distribution was strengthened both by the architecture of the toad's ventricle and by the failure of surface readings to account for the first deflection *R* of the axial electrocardiogram.

Internal Readings.

To obtain readings from the lining of the ventricle I have used a glass tube drawn out at the end to a bore of $\frac{1}{2}$ mm., and suitably curved for introduction through a slit in the wall of the left auricle. The end of this tube is plugged with the kaolin, the body of the tube is filled with copper sulphate.

Nine readings have been taken from five toads, the electrode being introduced well into the cavity, and these are given in Table IV, in which the earliest external reading is also given in an extra column. These readings, ranging from 0·0047 to 0·0572 second, are variable, as is to be expected in a musculature of this extent and complexity. With a solitary and unimportant exception, they are all earlier than the earliest external reading in the corresponding animals.

In three of the toads the contact was placed just inside the ventricle near the *A–V* ring. These additional readings are the very earliest obtained, 0·0005, 0·0012, and −0·0094, and approximated closely to the beginning of *R*. It should be stated that the intrinsic deflection in these three curves is relatively blunt at its origin, and the reading is therefore subject to greater error.†

The bluntness is ascribed to blending of extrinsic and intrinsic deflections, a phenomenon which was witnessed by MEAKINS, WHITE, and myself, when, in studying the dog's auricle, we led off from the region of the pacemaker.

I conclude, therefore, that the excitation wave spreads from the ring and passes outward to the surface through the wall of the heart.

---

* SANDERSON and PAGE estimated the rate at 125 mm. per second. CLEMENT estimates it at 320 mm. per second, but this value is clearly too high.

† But probably no more, however, than 0·0030 second.

TABLE IV.

Toad.	Figure.	Lowest external reading.	Number of external readings.	Internal readings.	Internal readings (near ring).
F	5	0·0668*	14	0·0377 0·0572	—
G		0·0543	18	0·0281	—
H		0·0391	4†	0·0047 0·0282	0·0005‡
J		0·0579	4†	0·0092 0·0132	0·0012§
K		0·0227	8†	0·0073 0·0254	– 0·0094§

* All readings are averages.

† Points specially chosen as probably including the earliest reading, *i.e.*, the central zone.

‡ Inside ring in front at left auriculo-ventricular border.

§ Inside ring in front near conus exit.

The Excitation Wave in the Ventricle as a Whole, and the Interpretation of the Curves of Extracardial Leads.

That the spread of the excitation wave in the toad's heart is in general from base to apex is a conclusion which is suggested by the form of electrocardiogram yielded by axial extracardial leads. The direction of the first and chief deflection R (fig. 1, and Plate 16, fig. 11) is such as to indicate primary base-negativity. This conclusion has also been drawn from observations upon the surface of the heart, though fallaciously, for, as we have seen, the surface basal muscle is, as a rule, the last to be activated. We have, then, this curious paradox; evidence of a general base-apex movement of the excitatory process, and yet a movement on the surface of the heart, over its cephalic half at all events, in a contrary direction. At this stage I would refer once more to the first hiatus between the surface readings and the initial phases of the axial electrocardiogram. *The surface excitation has not begun until the greater part of R is written; this phase of the curve, taken from an extracardial lead, results from the excitation of the deep musculature.*

In attempting further to elucidate the extracardial curves, it has seemed to me essential that they should be studied on a broader basis than has hitherto been attempted. A single lead from contacts placed away from the heart provides but an uncertain index of the direction of the excitation wave. It is necessary in the first instance, by adopting several leads, accurately to determine the electrical axis of the heart in a given plane.

Method of Estimating the Electrical Axis.—The method adopted to estimate the electrical axis in the present work has been that first described by EINTHOVEN, FAHR and DE WAART.* In using three leads, *I*, *II* and *III*, the contacts of which are arranged in the form of an equilateral triangle, the electrical potential developed by the heart is distributed on these lines according to the inclination of the electrical axis to them; and if the potential values expressed in the several leads are known, the direction of the electrical axis may be calculated trigonometrically.

Experimental Procedure.—Three contacts are arranged around the heart, being attached by stitches to the parietes of the unbroken body wall. The contacts are so placed as to form, so far as possible, the apices of an equilateral triangle. Two of the contacts lie on a line drawn horizontally across the body, usually on a level with the pectoral girdle, the third lies below the xiphisternum, near the heart's apex. Evidently the electrical axis will be calculated with the closest approach to exactitude when the triangle is exactly equilateral; but the error introduced by considerable deviations from the equilateral position is not very appreciable. Curves are taken from the three pairs of contacts, leading in the directions shown in the illustrations (figs. 3 and 4); and each of these curves, being of standard magnitude, is written simultaneously with a signal curve from a fourth lead (usually the floor of the mouth to a point on the right abdominal wall, see Plate 16, fig. 11). The three curves are measured on the comparator and plotted on millimetre paper, so that corresponding phases in time lie above each other vertically. A set of curves is accepted only if it obeys EINTHOVEN's formula throughout, that is to say, if, on any vertical line, the value of Lead *I* plus that of Lead *III* = the value of Lead *II*. The advantages of this procedure are manifold. The exclusion of curves which do not fit enforces accuracy of standardisation and of measurement; it eliminates all curves in which there are material errors in the estimation of time intervals or potential values. It checks in an admirable manner the factors of the apparatus employed; for a sufficiently quick response, uniformity of excursion with successive increments of potential within the working range, non-polarisability of the electrodes, are all essential to success. In brief, the fit of these charted curves assures us that each accurately portrays the current discharges which are tested, and that all are charted in correct time relations to each other. No material error in the calculation of angle is possible, except where, on a given vertical, all three curves nearly approach the base line of zero potential; at such points minute errors are of consequence and calculated angles may not be treated with the same confidence. Two such sets of plotted curves are given in figs. 3 and 4; and the relative values, expressed as potential, on successive 1/100 second time lines are given in the accompanying table† (Table V). The calculated angles formed by the electrical axis at the same time intervals are also given.

---

* 'Archiv f. d. ges. Physiol.,' vol. 150, p. 275 (1913).

† It will be noticed that small corrections have here and there been introduced into this Table; this has been done for the purpose of bringing them into perfect agreement with EINTHOVEN's formula.

TABLE V.

Toad B.

Seconds.	I.	II.	III.	Angle.
0·0100	0	1	1	90
0·0200	0	2	2	90
0·0300	0	4	4	90
0·0400	0	8	8	90
0·0500	-0·5	15	~~15~~ 15·5	92
0·0600	0	20	20	90
0·0700	-0·5	25	25·5	91
0·0800	-1	29	30	92
0·0900	-1·5	24	~~25~~ 25·5	93
0·1000	0	11	11	90
0·1100	0	0	0	
0·1150	0	-2	-2	-90
0·1200	0	-1	-1	-90

Toad C.

Seconds.	I.	II.	III.	Angle.
0·0100	-0·5	2	2·5	101
0·0200	-0·7	5	~~6~~ 5·7	96
0·0300	-1	13	14	94
0·0400	-3	24	27	96
0·0500	-3	25	~~29~~ 28	96
0·0600	-2	17	19	95
0·0700	-1	15	~~15·5~~ 16	93
0·0800	0	8	8	90
0·0900	0	7·7	7·7	90

Explanation of Table V.

In the "seconds" column and the columns marked *I*, *II* and *III*, the chief data of the charts of figs. 3 and 4 are tabulated.

The charts were originally constructed at twice their present dimensions, and consequently the ordinate values as given in columns *I*, *II* and *III*, and taken as millimetres, are double the present measurements of the chart, and double the actual measurements of the curves (see fig. 11).

The figures which are ruled out are the actual estimations; the figures placed beside them are the corrected values used in determining the angles.

In the fourth column, under "angle," the angle formed by the electrical axis with the horizontal (Lead *I*) is given (moving in clockwise direction from the right of the horizontal line).

Such deviations from exactitude as were present originally are not great; they are due to errors in measurement; as these are of insufficient magnitude to influence my conclusions, I have considered the attempt to reduce these errors (by re-measurement) less profitable than the construction of similar and additional charts from other animals.

The Direction and Movement of the Axis.—The electrical axis of the toad's heart, studied from one instant to the next, is remarkable in one respect. It lies almost in the line of the axis of the body and moves but little to one or other side of it. In Toad *A* it departed no more than 9° from the body axis; in Toad *B* no more than 3°, and in Toad *C* no more than 11°. It is for this reason that transverse leads (Plate 16, fig. 11, Lead *I*) are unfavourable; and for the same reason Leads *II* and Leads *III* are almost identical (fig. 11). During the stage in which *R* is written the electrical axis is inclined at an almost uniform angle of 90° to the horizontal; I interpret this observation as meaning that *during the whole of this period the movement of the excitation wave has an average and almost uniform direction from base to apex.* In some animals this direction is maintained throughout the whole period of the initial phases, in other and more numerous instances (Toad *B*, Table V) the direction of the axis becomes suddenly reversed, forming an angle of −90° or thereabouts with the horizontal; this change corresponds to the writing of *S* in Leads *II* and *III*; and I interpret it as being due *to a sudden reversal of the average direction in which the excitation wave travels, being now from apex to base.* To what is the change due? It is due, in my mind, to the last stage in the journey of the excitation wave, to the upward movement *at the base of the heart.*

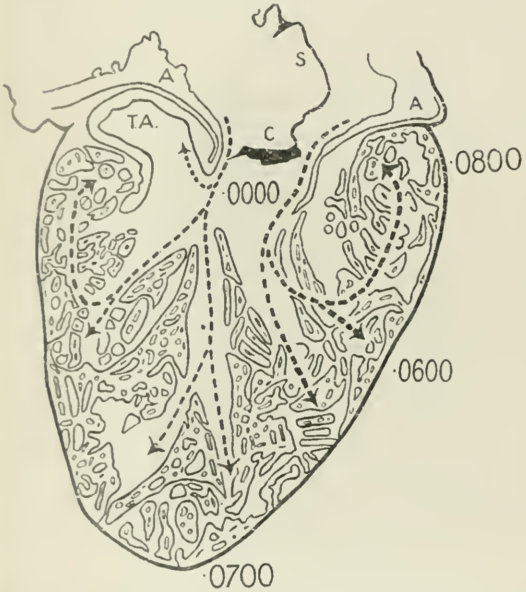


FIG. 6.—An outline drawing of a coronal section of a toad's heart, five times natural size. *A* = auricular muscle, passing into the *A–V* ring. *C* = endocardial cushion. *S* = auricular septum. *T.A.* = commencement of *truncus arteriosus.* The broken lines and arrows indicate the author's conception of the paths taken by the excitation wave. The readings (relative to *R*) in decimal points of seconds are intended to indicate the relative times at which the several muscular regions are activated.

My general hypothesis may be summed up diagrammatically. Fig. 6 represents a coronal section of the toad's ventricle, and illustrates the general arrangement of the musculature in the plane of our leads. The musculature of the auricle and ventricle does not meet at the surface *A–V* line, but the ring is prolonged as a tube for some distance into the ventricle; passing downwards it meets and fuses with processes of muscle which radiate towards the central surface zone and apex of the heart. While *R* is inscribed in the axial lead and the electrical axis is 90°, the excitation wave is spreading into the ventricle in many directions, but it is spreading more or less symmetrically and the average direction is down toward the apex. The slow onset of *R* is to be explained by the gradual and successive involvement of more and more fibres continuous with the ring. The trabeculæ first carry the excitation to the central surface zone of the heart. Later, the wave courses farther toward the apex and at the same time mounts to the base. If the basal passage outlasts† the apical, the general direction of travel becomes reversed and the electrical axis, as expressed by the axial lead, becomes −90°. This hypothesis accords with the general distribution of internal and surface readings. The actual expression of the change to −90° in an axial lead is the deflection *S*. I attribute *S* in axial leads in the toad to the final movement of the excitation wave over the base of the heart past the *A–V* groove. *S*, a deflection usually attributed to apical activity, appears to me in reality to be produced by a basal activity. That muscle activated at the base of the ventricle may be responsible for a dip in the curve, as opposed to a rise, has been shown already in the case of the *truncus arteriosus* (Plate 15, fig. 8, the dip marked *). It is not a question of the position of the muscle strip relative to the base or apex of the heart: it is a question of the direction in which it is activated, no matter where it lies. The axial electrocardiogram, as seen in an extracardial lead, must be regarded as a summation, an expression of the excitation of a large number of distinct muscle wedges, activated along separate routes. Each wedge brings, as a contribution to the total effect, its individual effect; the nature of its individual effect is controlled by two chief factors, the amount of muscle involved and the average direction in which the excitation wave passes through it.

Our conclusion in respect of *S* is justified by a comparison of the surface readings and Table I. In some axial curves from extracardial leads *S* is not developed; in the present series *S* was absent in Toads *C*, *H*, *J* and *K*. I have a sufficient number of surface readings in two of these animals: in Toads *C* and *K* the basal readings were of a lower order of magnitude than the apical readings. In Toad *A*, the basal and apical readings were similar, but descent below the base line was doubtful as the exact level of the latter could not accurately be determined. The remaining animals all presented *S* deflections of more or less prominence (*B*, *D*, *E*, *F* and *G*); these are the animals in which base readings came later than the extreme apical readings.

There seems, therefore, little reason to doubt that *S* in the axial lead from the toad's heart results from an upward passage of the excitation wave at the heart's base, and that the general distribution of the excitation wave is as I have depicted it in fig. 6.

There is, in the toad's heart, no evidence of a specialised conducting system in which the rate of conduction is peculiarly high. Evidence for this conclusion comes from three sources.

1. The rates of conduction, tested with artificial stimulation, are uniform over the whole ventricle, and are not dependent upon the architecture of the underlying structures.

2. The considerable time differences between separate readings from the lining of

---

† Or outweighs in virtue of the mass of muscle involved.

the ventricular cavity, in a given animal (Table IV), suggests a slow conduction rate over the lining.

3. The distance from the ring internally to the surface of the ventricular apex in the toads examined may be taken as a little less than 1 cm. The time taken for the excitation wave to travel over this path is approximately 0·0700—0·0800 second. The rate of travel along the lining is estimated therefore at about 100 mm. per second, a figure which does not differ materially from that calculated for the surface when the heart is excited.

Addendum.

TABLE VIII.—Extrinsic and Corresponding Intrinsic Deflections.

Toad A.		Toad B.		Toad C	
Extrinsic.	Intrinsic.	Extrinsic.	Intrinsic.	Extrinsic.	Intrinsic.
0·0209*	0·0667	0·0250	0·0872	–0·0012	0·0432
0·0235	0·0497	0·0410	0·0839	–0·0019	0·0404
0·0156	0·0676	0·0242	0·0848	–0·0043	0·0348
0·0181	0·0377	0·0400	0·0975	0·0275	0·0455
0·0059	0·0527	0·0347	0·0944	0·0094	0·0386
0·0163	0·0746	0·0201	0·0791	–0·0029	0·0376
0·0223	0·0526	0·0150	0·0810	–0·0092	0·0493
0·0077	0·0371	0·0161	0·0735	–0·0072	0·0523
0·0096	0·0538	0·0456	0·0863	0·0048	0·0471
—	—	0·0354	0·0718	–0·0015	0·0416
—	—	0·0219	0·0730	–0·0071	0·0422
—	—	0·0202	0·0711	–0·0016	0·0360
—	—	0·0313	0·0790	–0·0045	0·0388

* Each figure is an average of two readings.

Cantate No. 6 [illegible]

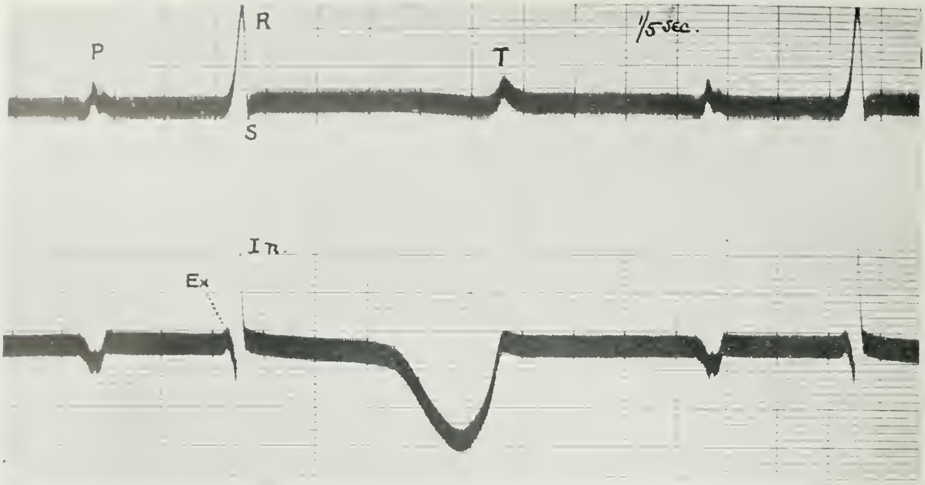


FIG. 7.

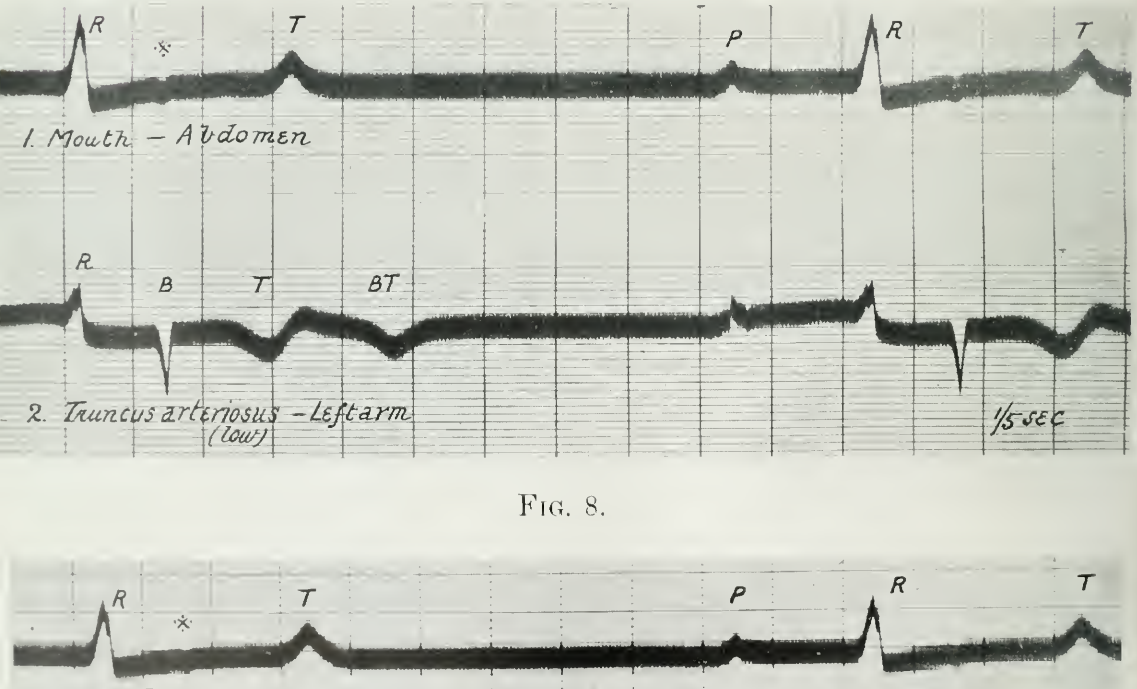


FIG. 8.

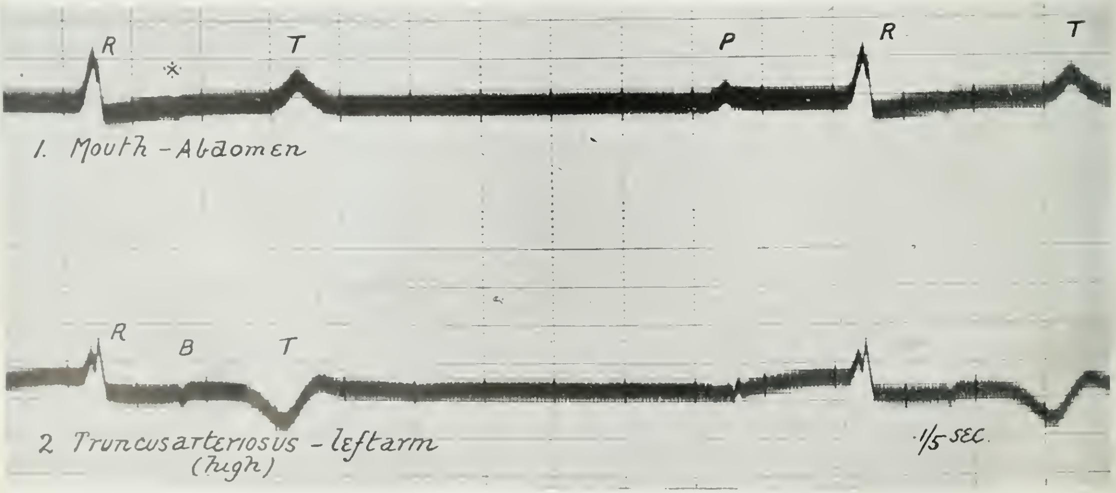


FIG. 9.

236 13/1

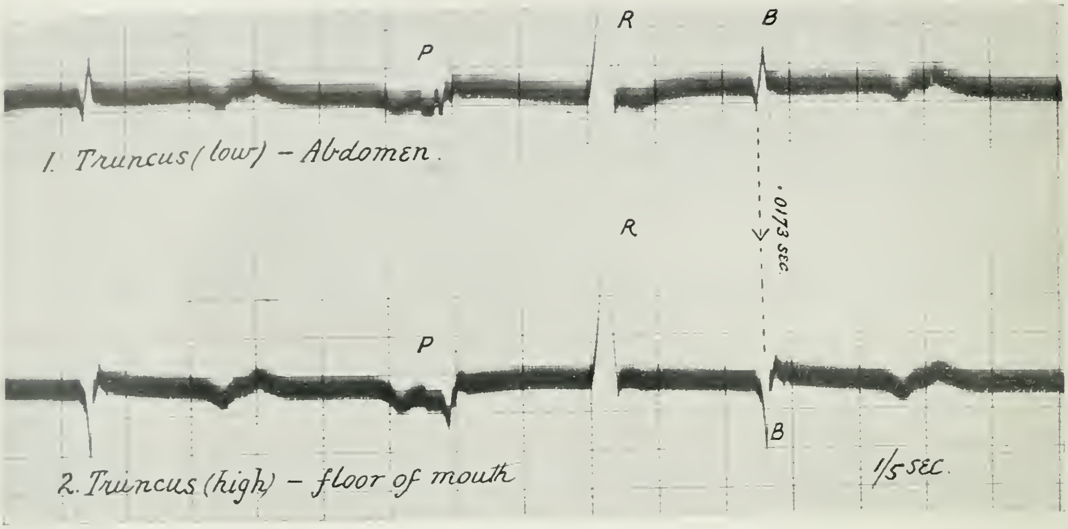

R
B
P
1. Truncus (low) - Abdomen.
·0173 sec
R
P
B
2. Truncus (high) - floor of mouth
1/5 sec.

Fig. 10.

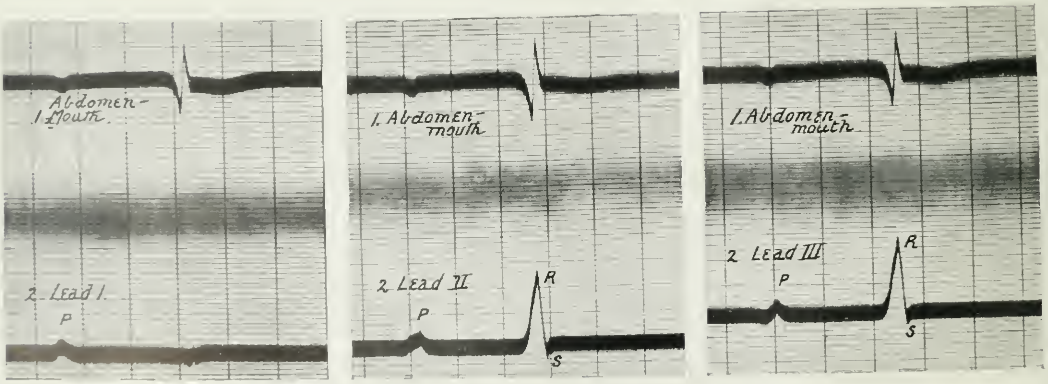

Abdomen-
1 Mouth.
2 Lead I.
P
1. Abdomen-
mouth
2 Lead II
P
R
S
1. Abdomen-
mouth
2 Lead III
P
R
S

Fig. 11.

EXPLANATION OF PLATES 15 AND 16.

NOTE.—The ordinate values expressed in the explanations of figured curves of this and the succeeding papers are approximate. In taking a curve from any lead for the purpose of charting it, three or more millivolts are introduced into circuit while such a curve is photographed. The extent of the excursion may be found to be a little more or less than the required number of centimetres. This error is eliminated in charting by introducing a corresponding correction.

Fig. 7.—Toad *D*. Simultaneous curves (nat. size): (1) Lead *II*, from right side of pectoral girdle to mid-abdomen: (2) base of ventral surface of ventricle to body wall. The lower curve shows diphasic extrinsic deflections (*Ex.*) followed by a steep intrinsic deflection (*In.*). The reading of the intrinsic deflection is obtained by estimating the interval between the summit of *R* in the upper curve and the commencement of the intrinsic deflection in the lower curve, and subsequently relating it to the commencement of *R*. The vertical lines represent 0·2 second intervals.

Fig. 8.—Simultaneous curves (nat. size). (1) Standard lead from mouth to abdomen; this shows *R* and *T*, the ventricular summits, and a little downward notch marked *, which is due to the passage of the excitation wave through the *truncus arteriosus*. (2) Lead from *truncus arteriosus* to left arm region; this shows extrinsic deflections, which may be recognised as corresponding approximately to *R* and *T*, and intrinsic deflections *B* and *BT*. The *upstroke* of *B* corresponds to the arrival of the excitatory process under the *truncus* contact, *BT* to its subsidence at the same point. The *downstroke* at *B* is an extrinsic effect of the truncus itself.

Fig. 9.—Simultaneous curves from the same animal. (1) The top lead has been maintained. (2) The *truncus* contact has been moved off the musculature of the *truncus* to the commencement of the artery (for actual contact see fig. 4). This curve is consequently different from that of fig. 8. *R* and *T* and another small extrinsic deflection *B* remain, but the intrinsic deflections, *i.e.* the greater part of *B* and the whole of *BT*, have disappeared.

Fig. 10.—Simultaneous curves (nat. size) showing the method of estimating conduction rates. Two contacts are placed on the *truncus arteriosus*, above and below, and each is paired with a separate and indifferent contact on the body wall. The time interval between the intrinsic deflections (first sharp upstroke, *BB*) of the *truncus* is estimated. In this instance it amounted to 0·0173 second. The *truncus* contacts lay 2 mm. apart.

Fig. 11.—Three simultaneous curves ($\frac{2}{3}$ nat. size) from Toad *B*. In each photograph the upper curve is a standard curve from which to measure. The lower curves are taken from Leads *I*, *II*, and *III* (see fig. 3) respectively. The sensitivity of this string was such that 1 centimetre's excursion (10 scale divisions) corresponded to 1 millivolt. Time marker = 0·2 second. These three curves were utilised in constructing fig. 3 and Table V. Toad *B*.

Part II.—*The Tortoise Ventricle.*

[Plate 17.]

Observations have been carried out upon female tortoises of the species *Testudo græca.* They were conducted in the months of May and June, the tortoises being kept in the sun. In an experiment the head was severed from the neck and, the vessels being ligatured, the spinal cord was pithed. The animals were fixed in a sand-bath, the temperature of which was raised. The plastron was trephined in its centre and the cephalic portion removed by diverging saw cuts running forward from the trephine hole.

The leads, arranged around the sides of a triangle, were taken at this stage and before the pericardium was opened. Two contacts were sewn symmetrically over the pectoral girdle, the third was placed on the middle line to the caudal side of the heart. In taking the three leads a separate exocardial lead was used as standard; this lead was from the neck to a point on the caudal and right side of the heart.

Subsequently, the pericardium being opened, direct leads were obtained from the front of the ventricle in the usual fashion, Lead *II* being now employed as the standard.

The distribution of the excitation wave in the tortoise heart in many respects so closely resembles that of the toad that a very full description of it is not considered necessary. This heart serves, however, to illustrate or emphasise important principles.

I find the beat of the tortoise heart, once the pericardium is opened, to be very unstable; it is very sensitive to changes of temperature and may alter appreciably in response to change in the animal's posture. The last change appears to result from the relation of the pericardial fluid to the organ, and may, I believe, be the effect of local temperature changes. My animals have generally been inclined so that the cephalic end of the carapace lay some few inches higher than the caudal end: the pericardial fluid consequently surrounded the apical portions of the ventricle.

In axial exocardial leads the electrocardiogram shows a prominent deflection *R* (Plate 17, fig. 5) and an isoelectric line, or a line which dips as it proceeds, continued into a downwardly directed *T* deflection. As a rule there is no true *S* deflection, but this may be seen in greater or lesser degree, especially before the pericardium is opened. If *S* is prominent then *T* is upright, a relation not without importance, but one which must be left for later and separate research.* These variations are described because the diagrams of surface distribution shown in fig. 1 are, without exception, from hearts showing no *S* deflection and an invert *T*, and it may be open to question whether or not this type represents the most habitual and natural heart-beat in the tortoise. The instability of the tortoise heart clearly emphasises the importance of obtaining, as an accompaniment of a series of direct surface readings, standard curves of constant outline, for it is evident to me that any change in the form of the standard curve is accompanied by a change in surface distribution. Observations which bear out this statement deserve illustration, for the fact is of consequence. It will, however, be convenient to deal with this matter more fully at a later stage.

---

* There is a similar relation between *S* and *T* in the toad's heart, *T* being upright whenever *S* is prominent and *T* being inverted when *S* is absent. Evidently these facts may help us to recognise the meaning of *T* and its inversion

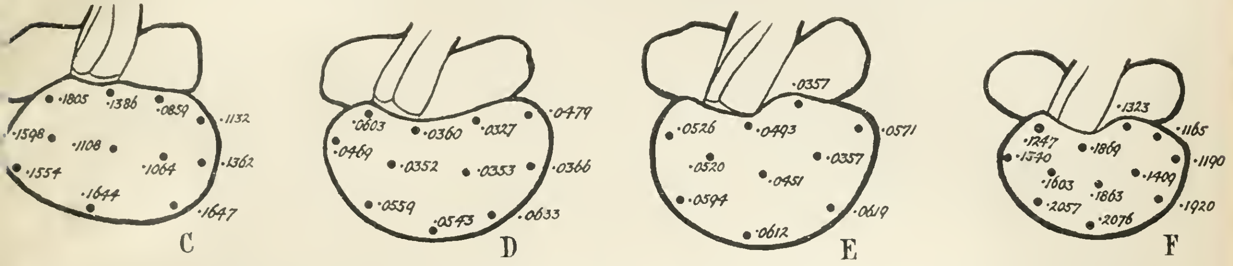


Fig. 1.—Four natural-size outline diagrams to scale of the hearts of Tortoises C–F, showing the surface distribution of the excitation process.

Table I.

Tortoise.	Lead *II.*				Surface readings.		Lining readings.			Average length of intrinsic deflec-tions.	Temp.	Heart rate.
	R.	Summit of *R.*	End of *R.*	End of *S.*	Earliest.	Latest.	Near ring.	Farther in.	Farthest in.			
B	0·0000	0·0750	0·1300	None	0·0443	0·0826	0·0102	0·0362 0·0432	0·0654	0·0250	20–21°	26
C	0·0000	0·1345	0·2137	None	0·0859	0·1805	—	—	—	0·0155	21–22°	33
D	0·0000	0·0546	0·0932	None	0·0327	0·0633	0·0145	0·0314	—	0·0158	29–30°	39
E	0·0000	0·0593	0·1028	None	0·0357	0·0619	0·0144	0·0269	0·0600	0·0234	27°	52
F	0·0000	0·1446	0·2732	None	0·1165	0·2076	—	—	—	0·0293	20°	24
G	0·0000	0·0496	0·0900	None	0·0319	0·0544	0·0115	—	0·0387	0·0177	21–30°	32

When the pericardium has been opened, the distribution of the excitatory process over the ventral surface is as illustrated by the outline diagrams of fig. 1. It is not dissimilar to that found in the toad. It is the rule that the apical portion of the ventricle is latest, the base and central region, or the central region, earliest. From time to time a high reading is obtained from the region of the base in the neighbourhood of the *truncus arteriosus.* The right margin is sometimes activated at a later time than the left margin. The whole ventral surface is activated in two or three hundredths of a second (fig. 1, Tortoise *D*, *E*) when the initial phases of the axial electrocardiogram are relatively short (Table I) and the heart beat active. The completion of surface activation takes longer when the initial phases are of greater duration (Tortoise *C*, *F*). The arrangement and values of the readings, compared with the surface conduction rates in the same hearts (Table II) preclude the idea of a simple surface spread and suggest, as in the toad, a distribution from within outwards. As in the toad's heart the time period of the initial phases is only partially covered by the surface readings. Thus in Tortoise *B* (fig. 2) the initial phases of the three leads shown in the corresponding diagram have a total duration

TABLE II.—Rates of Conduction.

Tortoise and temperature	Interval.	Distance.	Rate	Direction.	Distance of stimulating electrodes.
	secs	mm.	mm. per sec.		mm.
B (20–21)	0·0420	7	169	↑	—
B	0·0447	7·3	163	→	3
	0·0467	7·3	156	→	12
B	0·0566	8·5	150	←	5
	0·0567	8·5	150	←	14
C (21–22°)	0·0987	9	91	←	7
C	0·0807	10·5	130	↖	6
C	0·0753	7	93	↗	3
	0·0739	7	95	↗	11
D (29–30°)	0·0308	6	195	↖	4
D	0·0229	4	175	↗	11
E (27°)	0·0256	7	273	↗	4
E	0·0291	6	206	↗	4
E	0·0241	6	249	↗	10

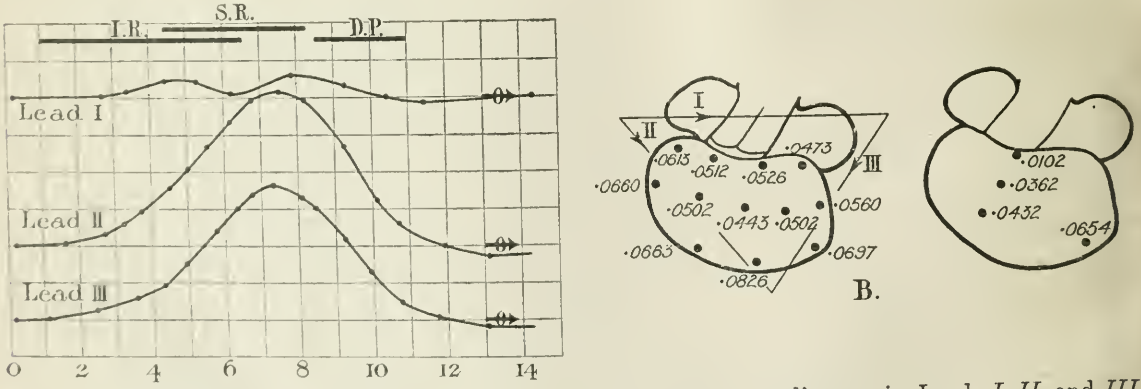


FIG. 2 (Tortoise B).—A chart showing the initial phases of the electrocardiogram in Leads *I*, *II*, and *III*. Ordinates, 3 cm. (6 scale divisions) = 1 millivolt; abscissæ, 1 cm. (2 scale divisions) = 0·02 second. The left-hand diagram shows the surface readings for the same heart and the relation of the three leads of the chart to the ventricle in the same animal. The right-hand diagram shows four readings from the lining of the same ventricle, taken approximately from the points shown. All the readings are related to the 0 line of the chart. *I.R.* = period covered by internal readings; *S.R.* = period covered by surface readings; *D.P.* = period covered by developing potential difference.

of some twelve or thirteen hundredths of a second, the superficial readings for the ventral surface corresponding to the period covered by the line *S.R.* They correspond to the last half of the rise of *R* and to its summit. There is, as in the toad, an initial and a terminal hiatus. The terminal hiatus is to be explained chiefly by the time taken in the full development of the charge, which amounted, as estimated from the length of the intrinsic deflections, to at least 0·0250 second in this animal (fig. 2, line *D.P.*). The initial hiatus is to be accounted for by the early activation of the muscle, which lay deeply. The tortoise heart is more manageable than that of the toad, and the positions of internal contacts are more readily determined. The earliest readings are taken from that portion of the lining which borders the *A-V* ring and, as the contact is moved towards the apex of the ventricle, so the readings are later. In fig. 2 a second diagram, that to the right, shows the positions of four internal contacts and the corresponding readings for Tortoise *B*. They cover practically the whole of the initial hiatus and overlap the surface readings to a considerable extent (fig. 2, line *I.R.*). The earliest readings obtained lay 0·0102 second after the earliest trace of *R*, the latest reading some 0·0654 second after the same phase of the initial deflections in the axial electrocardiogram. It is clear from a diagram of this kind that the initial phases correspond to the activation of the ventricle as a whole; a rule which applies to all vertebrate hearts, as we shall eventually discover. In the tortoise, the rule is further illustrated by Table I, where earliest and latest readings are given side by side with the length of the initial phases of axial curves; absolute correspondence is not to be anticipated, for the whole surface and lining is not investigated in a given animal. But the Table shows an unmistakable relation between the length of the initial phases and the duration of the activation process, as estimated from direct readings. Briefly, the axial curve in its initial phases is a summation of those electrical effects which signal the involvement of the ventricle in all its parts.

Thus the duration of the initial phases is controlled by the speed with which the excitation wave is distributed, and in beats of natural type the speed is mainly governed by the conducting power of the muscle elements. In support of the latter statement it may be noted that where the estimated conduction rate approaches 200 mm. per second (Table II, Tortoise *D* and *E*) the initial phases have an approximate duration of a tenth of a second. Where the conduction rate approaches 100 mm. per second (Table II, Tortoise *C*) the initial phases last approximately two-tenths of a second; where, as in Tortoise *B*, the conduction is of intermediate rate, the length of the initial phases is also intermediate.

As the duration of the initial phases is controlled by the speed of distribution, so the form of these phases is controlled by the paths of distribution. The direct readings convey an imperfect idea of these paths, though indicating a general resemblance to those of the toad's heart. The electrical axis and its movement complete our information. It is unnecessary for our purpose to calculate the exact electrical axis for the heart of the tortoise: for as the chief differences in potential are found between the contacts forming Leads *II* and *III*, the curves of these leads being of considerable magnitude and almost identical in outline, while the curve of Lead *I* is always diminutive, so we may conclude that the electrical axis is almost in the axis of the body throughout the whole of the initial phases. And, the chief of these phases being upward, the angle formed with our base line is constantly in the neighbourhood of 90°. It may be that it turns abruptly to −90°, as in the frog, when an *S* develops: but in most of the studied instances a straight base-apex spread has been maintained. In a solitary and peculiarly instructive experiment this mode of spread was temporarily in abeyance. This experiment is illustrated by fig. 3. The curves of the triangular leads are charted, the distribution on the surface

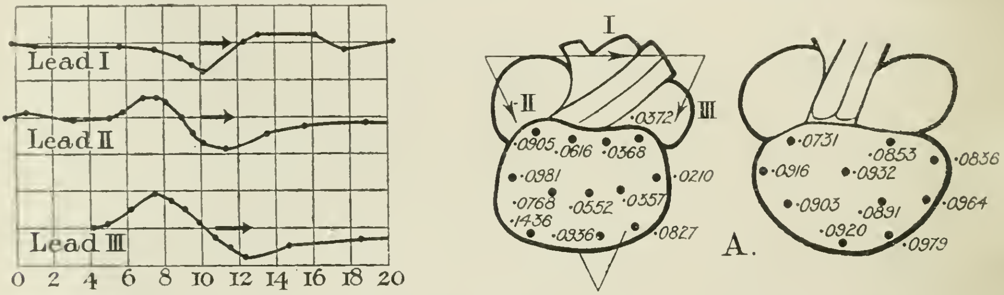


FIG. 3 (Tortoise A).—A chart showing the initial phases of the electrocardiogram in Leads *I*, *II*, and *III*. Ordinates, 3 cm. (6 scale divisions) = 1 millivolt: abscissæ, 1 cm. (2 scale divisions) = 0·04 second. The left-hand diagram shows the surface readings corresponding to the chart and the leads employed in obtaining the curves of the chart. The right-hand diagram shows a later and more normal surface distribution of the excitatory process in the same animal, when the curves of the triangular leads had resumed their customary outlines.

TABLE III.—Tortoise A

Second	*I.*	*II.*	*III.*	Angle.
0·0400	−1	−1	0	? −150°
0·0500	−1	0	1	? 150
0·0600	−1	2·5	~~4~~ (3·5)	106
0·0700	−2	5	~~8~~ (7)	106
0·0800	−3	5	8	112
0·0900	−5	0	5	150
0·1000	−7	−7	0	−150
0·1100	−5	−8	−4	−124
0 1200	−1	−8	−7	−97
0·1300	2	−6	−8	−76
0·1400	2	−4	−6	−71
0·1500	2	−3	−5	−67
0·1600	2	−2	−4	−60

is shown in the left-hand diagram. Now the spread on the surface is unusual in several respects. First, it is unusually slow; second, the left margin is supplied earlier than any other region of the ventral surface; and third, the readings show an orderly sequence from left to right. Unquestionably, this mode of spread is unnatural; it was observed in the first tortoise examined, and before the influence of temperature had been fully recognised (the actual temperature of the beast was 18°). I imagine that the impulse was impeded in its spread through the *A–V* ring, being confirmed in this supposition by finding an unusually prolonged *P–R* interval in the associated curves, and that the impulse spread to the ventricle entirely or chiefly through the left sector of the ring. From this margin it would follow the musculature, and proceed across the ventricle from left to right, down the left margin and along the broad apex from left to right, to rise finally, perhaps, on the posterior surface and extreme right margin. When we examine the charted curves and the calculated electrical axis (Table III) we discover corresponding features. The initial phases are exceptionally long. At the very beginning, the angles are of doubtful value and sign, but from 0·0600 second* onwards, they are trustworthy. The path of spread which is indicated is at first from above downwards and slightly from left to right (106° to the horizontal); it remains at this angle for a while and subsequently changes in a regular clockwise fashion through 194°, the predominating direction being from left to right. The general spread, so far as the whole musculature was concerned, was at first from base to apex, later from left to right, and finally from below upwards. Whether a local conduction defect in the ring is a sufficient explanation of this unusual distribution need not detain us; I use the experiment to illustrate the close connection between the order of the direct readings and the direction and change of the electrical axis. And this experiment is of greater value, because, maintaining the animal in a warm atmosphere, the usual type of curves from extracardial leads subsequently prevailed.† A second series of surface readings was obtained, and is shown in the right-hand outline diagram of fig. 3. The general arrangement of these later values was the usual arrangement for the tortoise.‡

The investigation of the tortoise ventricle produces no evidence that there is in this organ any special tissue which conducts more rapidly than the surface muscle fibre. In this respect the heart is like the toad's, and my reasons for this statement are similar to those enumerated for the toad's heart. There is the additional argument, in the case of the tortoise heart, that in estimating the rate of conduction, the distance of the stimulating electrodes from the proximal contact is without influence upon the result. In the dog's heart, if the stimulating electrodes are at a distance, the excitation wave has time to traverse the thickness of the muscle wall, and entering the Purkinje arborisation is carried rapidly to the two contacts between which the conduction rate is tested. Removing the stimulating electrodes from a point near to, to a point far from, the proximal contact, results in a notable increase of conduction rate between the two contacts in this animal. In the tortoise the conduction rate is unaltered by this procedure (Table II), and I conclude from this and the remaining arguments (see Part I) that a system having special powers of conduction does not exist in this animal.

---

* The earliest surface reading is 0·0210 second; allowing 0·0250 second for the development of the discharge, we arrive at the figure 0·0461 second.

† These curves were of the type shown in fig. 2, but the heart being still a little unstable when the three leads were taken, and the series failing as a consequence to show a perfect fit, I do not reproduce a corresponding chart.

‡ The values of this diagram are perhaps not strictly compatible with each other, because the heart beat was still somewhat unstable; however, it was sufficiently stable to render them compatible in a general sense.

The chief difference between the distribution in the toad's heart and in those tortoise hearts which I have examined is to be found in the relatively early activation of the base of the ventricle in the latter. This feature is entirely compatible with the absence of an *S* deflection in the extracardial axial leads of the corresponding animals, and confirms the view that *S*, appearing in an axial lead from the toad's heart, is due to the late activation of basal muscle.

In the tortoise ventricle the fusion of the ring musculature with the ventricular wall at a level relatively nearer to the base than pertains in the toad's heart seems a sufficient explanation of this minor difference in the mode of spread in the two beasts.

EXPLANATION OF PLATE 17.

Figs. 4, 5, and 6 (Tortoise *G*).—In each of these photographs the upper curve is the standard curve from the neck to a point outside the right and caudal aspect of the ventricle, the lower curves are from the leads *I*, *II*, and *III* of the triangle.

Ordinates of lower curves, 1 cm. = 1 millivolt; abscissæ = 0·2 second.

Figs. 7, 8, and 9 (Tortoise *G*).—Fig. 7: A direct lead from the lining of the ventricle near the *A–V* ring and an indifferent point on the body wall, taken simultaneously with Lead *II*. Fig. 8: Similar curves, the direct lead being from the ventricular surface near its centre. Fig. 9: Similar curves, the direct lead being from the lining of the heart near the ventricular apex.

In each direct lead an extrinsic deflection (*ext.*) and an intrinsic deflection (*int.*) is shown. The extrinsic deflection is very deep in fig. 9, but the intrinsic deflection rises from it sharply.

Ordinates of direct curves, 1 mm. = 1 millivolt; of lower curves, approximately, 1 cm. = 1 millivolt; abscissæ, 0·2 second.

vol. 6. [illegible] No. 6 [illegible]

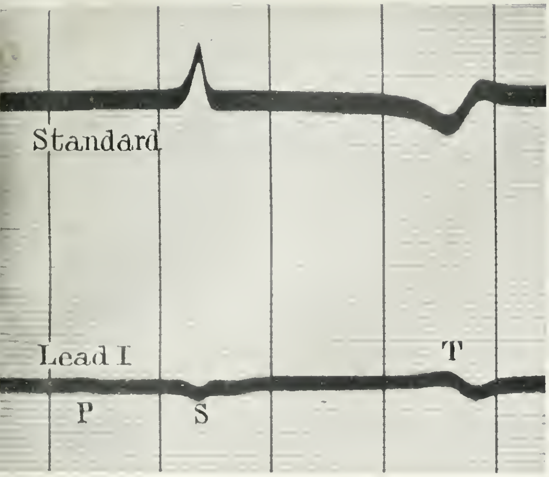


Fig. 4.

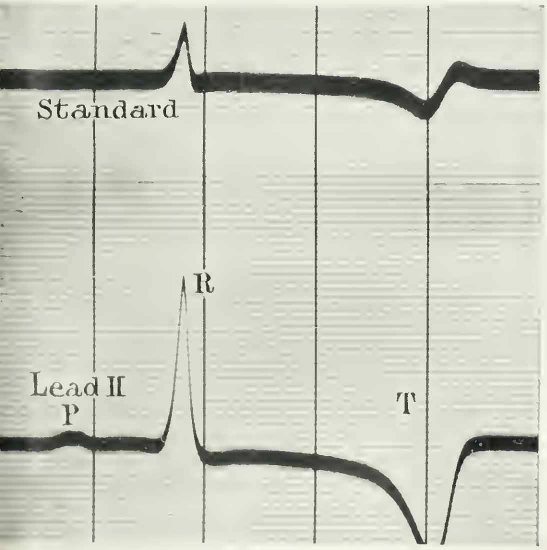


Fig. 5.

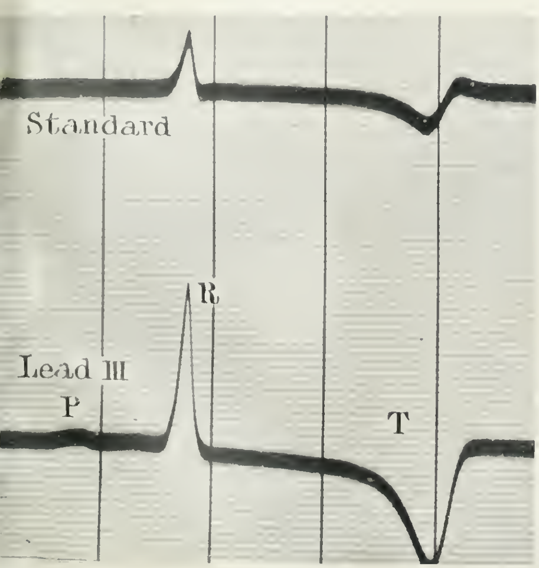


Fig. 6.

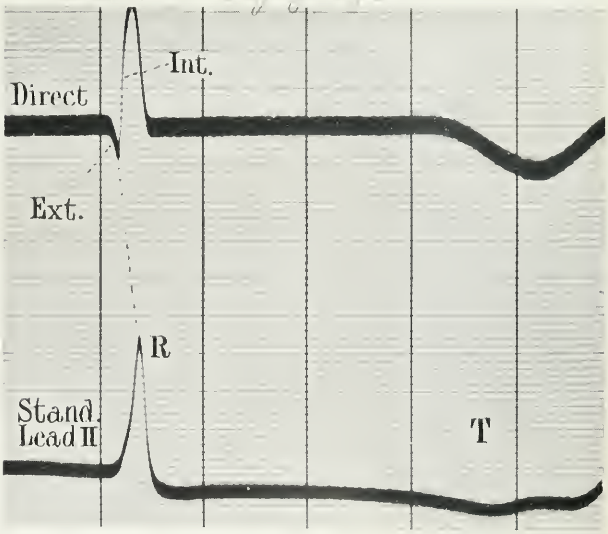


Fig. 7.

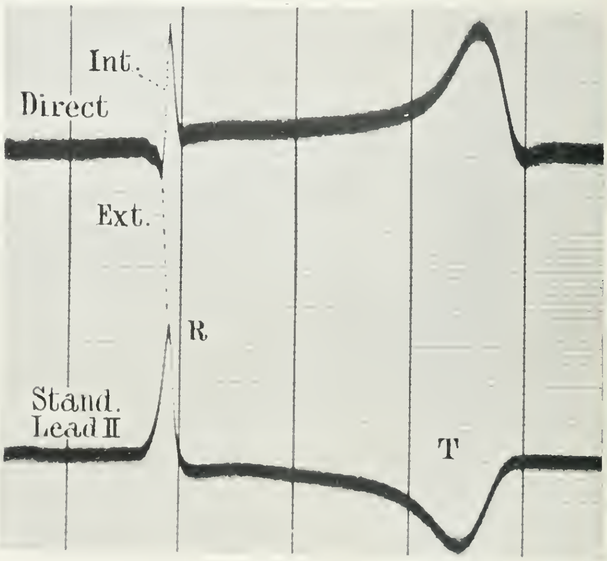


Fig. 8.

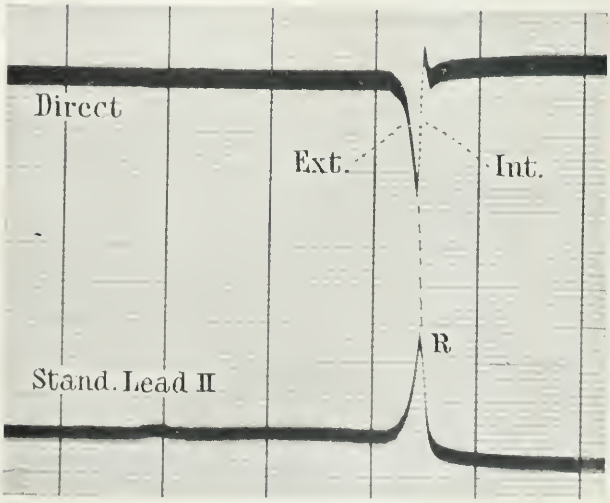


Fig. 9.

Part III.—*The Dog's Ventricle.*

[Plates 18–20.]

THE DUALITY OF THE VENTRICULAR ELECTROCARDIOGRAM.*

In recent observations upon the dog's ventricle Rothschild and I advanced experimental evidence which we believe shows conclusively that the excitation wave is distributed to the mammalian ventricle through the arborisation of the auriculo-ventricular bundle and its branches.† In our paper we published a number of maps exemplifying the distribution of the excitatory process over the surface of the ventricles in the dog. The readings of commencing surface activity, as there portrayed, are related to one another in a manner which must now receive emphasis. So far as the right ventricle is concerned, the earliest sign of activity appears over what we term the central region of the ventral surface, a region situate close to the ventral attachment of free wall to septum. As the readings are traced from this region towards the auriculo-ventricular groove, they become gradually later in point of time; they show a similar increase in value as they are traced towards the actual attachment of the septum. Over the whole of the right ventricle, neighbouring readings either approximate to one another very closely or show a gradual succession. The last statement applies precisely to the surface of the left ventricle also. But the statement does not apply to the ventricles treated together. Speaking especially of the ventral surface, the readings on one or other side of the septum diverge notably from each other, the higher readings being over the left ventricle. This is no doubt in great part due to the greater thickness of muscle on the left side and to the consequent delay in the arrival of the excitation wave at its surface; but the relatively abrupt change is evidently conditioned also by the isolation of the spread to the free walls of the right and left ventricles. That is to say, the whole free wall of the right ventricle receives its supply from the right bundle division, the whole free wall of the left ventricle is supplied by the left bundle division; during the natural heart beat the excitation wave does not spread from the border of one ventricle to the neighbouring border of the other ventricle. The two waves spreading on right and left sides meet and end in the septum. This conclusion, which is alone compatible with the normal surface distribution whose study first prompted it, is completely borne out by comparing the surface readings before and after section of a single large branch of the distributing tracts on right or left side. We showed in our paper that if the large septal branch of the right division, which supplies at least the whole ventral surface of the right ventricle, is divided, the surface readings over the left ventricle remain unaltered relative to each other; but that the readings over the ventral surface of the right ventricle are all disturbed, being later in point of time and of such values as to show a spread of the excitation wave from left ventricle to right. Briefly, the described procedure abolishes that feature of the physiological heart beat which is so noteworthy, namely, the strict confinement of spread through a given bundle division to the corresponding ventricle.

---

* A preliminary report of these observations appeared in the 'Proceedings of the Physiological Society,' March 13, 1915.

† 'Phil. Trans.,' B, vol. 206, p. 181 (1915).

During the course of the present experiments these observations have been repeated and extended; and it may now be stated in more general terms that if one or other of the two chief divisions of the $A-V$ bundle is interrupted, the spread to the contralateral ventricle is unaffected, while the spread over the homolateral chamber is profoundly altered; the direction of travel subsequent to the injury being from the side contralateral to the lesion towards and over the side homolateral to the lesion.

In view of these observations I conclude that the normal axial electrocardiogram is in reality a *bigram*, a dual picture of the curves of right and left ventricle respectively; the *bigram* consists, according to this hypothesis, of the algebraically summated *dextrogram* and *levogram*.

My next endeavour has been to bring forward proof of this proposition, and the following experiments have been directed to that end.

We may pause for a moment to consider the curves obtained by interrupting the right or left bundle division, respectively. A lesion of the right stem produces in Lead *II*, a curve of the form shown in the central curve of Plate 18, fig. 12.* The change from the natural type (Plate 18, bottom curve of fig. 12) is conspicuous and characteristic. We may term such curves *levograms*, with a single qualification. They are true levograms in their initial phases only. While the initial phases are being inscribed the excitation wave is spreading, as observation upon surface distribution clearly shows, along perfectly natural paths in the left ventricle, while the whole right ventricle remains inactive. But after a time interval of 0·0350 to 0·0400 sec. has elapsed,† the spread is complete throughout the left ventricle, and has commenced its abnormal course through the right ventricle; from this point onwards therefore the curve is no longer a true levogram.

Precisely similar arguments apply to the curves yielded by lesions of the left bundle division (Plate 18, top curve of fig. 12). In their initial phases they are true expressions of the dextrogram. By using a special form of clamp‡ it is possible to obtain in one and the same animal the curves corresponding to right and left bundle branch lesions, by alternately compressing these branches. The experiment is not easy to perform, and is precarious to this extent, that it is not possible subsequently to identify the tissue areas involved in the compression. Gentle pressure is exerted, working from the ventral towards the dorsal border of the septum at its base, until abruptly the normal axial curve vanishes, and is replaced by a curve which is recognised as characteristic of a right branch lesion. The necessary observations upon this disturbance being completed, pressure is relaxed and recovery is awaited; when the normal curve is resumed—the recovery is gradual—the forceps are carried a little more dorsalwards, and the compression re-applied. In a successful experiment, a curve characteristic of a left branch lesion results.

---

* That the curves correspond to the lesions named is for the moment partly assumed; the evidence will be given in detail at a later stage of this report.

† From the commencement of R.

‡ The clamp adopted has been described by MEAKINS, the curve of the instrument being modified slightly to throw its jaws more towards the ventral surface of the heart.

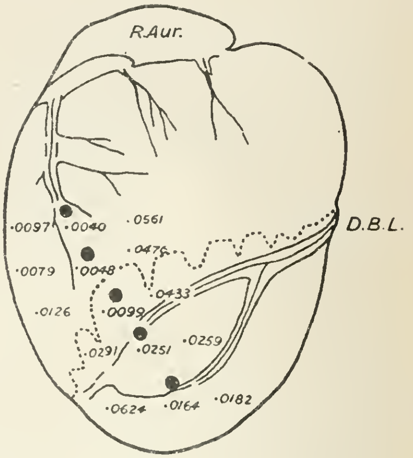


Fig. 1.—Outline drawing to scale (2/3 natural size) of the heart of Dog H.H. *D.B.L.* = descending branch of left coronary artery. Five contact points were used in this instance, curves being taken from each separately paired with a constant chest wall contact and simultaneously (*a*) with the natural electrocardiogram from Lead *II* (the corresponding readings for the surface points are placed immediately below the latter), (*b*) with the levogram (the corresponding readings are placed to the right), and (*c*) with the dextrogram (the corresponding readings are placed to the left). The dotted line represents the right attachment of the septum.

Thus three sets of curves are obtained: the normal, and those which include the initial phases of the true *levogram* and *dextrogram*. All curves are standardised, so that 3 cm. of excursion are equivalent to 3 millivolts of potential.

The next step is the orientation of these curves so that they may be charted with corresponding phases in time lying vertically above each other. The procedure is as follows: the ventral surface of the heart being exposed, the natural curve from Lead *II* is used as a standard of measurement; and, simultaneously with this, successive curves are taken by means of direct leads* from suitable points on the surface of the right and left ventricle (see contact points in fig. 1). The present illustration is one of two examples.

The times at which the excitation wave appears at these points, relative to *R* in the standard lead, are estimated (these readings are placed immediately below the contact points in fig. 1).

The clamp is now applied and the curve corresponding to a right branch lesion obtained; fresh readings are then taken from the original contact points, using the small initial upstroke of the levogram as standard (these readings are placed to the right of contact points in fig. 1). After recovery a third series of readings is taken, using the upstroke of the dextrogram as standard (these readings are placed to the left of the contact points in fig. 1).

---

* The direct lead is from a contact with the surface of the heart and an indifferent point on the chest wall, as hitherto adopted.

Now if the readings of surface points for the left-sided beat are compared with those of the natural beat, it will be seen that the order over the *left* ventricle is unchanged, and that the actual values change only slightly. The same statement applies to the surface of the right ventricle when the natural and right-sided beat are compared. Over the ventricle homolateral to the lesion the values are greatly raised and are arranged in a fashion indicating spread from the undisturbed side.* The slight change in the actual readings over the contralateral surface is due to alteration of the standard of measurement, for it varies in the same direction and by approximately the same amount in each instance.

In charting, I take the beginning of *R* in the natural curve of Lead *II* and place it on the 0 time line (fig. 2), subsequently plotting the whole of the initial phases of the bigram. In charting the initial phases of the levogram, I commence the small initial upstroke of this curve by plotting it relative to *R*; this point in time is arrived at by averaging the differences in the two sets of corresponding readings over the left ventricle. Thus *R* is placed on the 0 time line for the heart of fig. 1; the differences, 0·0259 − 0·0251 = 0·0008, and 0·0182 − 0·0164 = 0·0018, give an average of 0·0013. The initial upstroke of the levogram is charted as preceding the upstroke of *R* in the natural curve by 0·0013 second (see fig. 2).

Similarly the average difference between the two sets of readings over the right ventricular surface, corresponding to natural and right-sided beats, is used in charting the dextrogram. Thus, 0·0097 − 0·0040 = 0·0057, 0·0079 − 0·0048 = 0·0031, and 0·0126 − 0·0099 = 0·0027, the average difference being 0·0038; the upstroke of the dextrogram is charted as commencing before *R* by this time interval (see fig. 2).

Thus I am able to chart from one animal standard curves corresponding to the natural beat (fig. 2, *N*), the left-sided beat (fig. 2, *Lf*), and to the right-sided beat (fig. 2, *Rt*), and to place them with corresponding time phases on the same vertical line. If now the left- and right-sided beats are summated algebraically, a calculated curve is obtained (fig. 2, *C*). This curve is to all intents and purposes a duplicate of the natural curve both in respect of its time relations and in respect of the voltages expressed by its different deflections. Even the notch on the upstroke of *R* is clearly and accurately repeated in the illustration. It departs from the form of the natural curve where it may be expected to depart from it, namely, at 0·0350 – 0·0400, for after this time interval the left- or right-sided curves no longer represent pure levogram or pure dextrogram.

Thus it is shown that in its initial phases the natural electrocardiogram is a composite curve, built up of the superimposed effects of right and left ventricle respectively. It is a *bigram* comprising an algebraically summated *dextrogram* and *levogram*.

---

* The unaltered relation of the readings on the side unaffected by the lesion and their characteristic increase over the affected side is a sufficient check upon the nature of the curves obtained. It is not only upon the appearance of curves of characteristic shape, but upon these readings, that I base the assumption that conduction in the bundle branches has been successfully and alternately hindered in a given experiment of the kind now described.

This initial step in the analysis of the axial electrocardiogram is important. Clearly, the further analysis should consist in separate analyses of dextrogram and levogram respectively.

Meanwhile we may point to the origin of certain deflections in the bigram in terms of left or right ventricle. *Q* appears, so far as these observations go, to arise in the dog from the left ventricle in Lead *II*. Where *Q* is absent in the bigram it is absent in the levogram. *R* is in the main a right-sided event, but may be added to by the curious and almost constant hump (marked by an asterisk in fig. 2) of the levogram. *S* is decidedly the result of the preponderating downstroke of the levogram.

The analysis is also valuable in explaining variations in the form of the natural curve. Evidently slight variations in the time relations of dextrogram and levogram will profoundly modify it. Thus the hump of the levogram, falling early, notches and broadens the upstroke of *R*; falling later it blends with and raises the summit of *R*. Of other possible variations I shall speak more fully at a later stage.

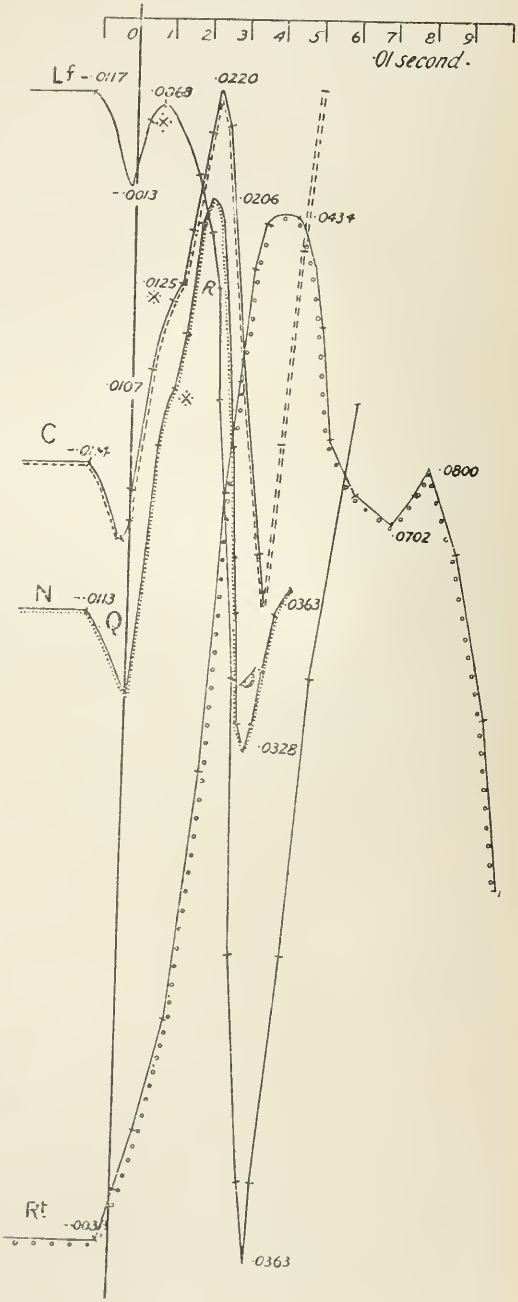


FIG. 2.—A chart of four curves. *Lf* is the levogram, *C* is the calculated bigram, *N* is the actual bigram, and *Rt* is the dextrogram of Dog H.H. All the curves are plotted in relation to *R* of the natural bigram, and this stands on the 0 line. The calculated bigram (*C*) has been constructed by algebraic addition of the values found on corresponding vertical lines in dextrogram and levogram. It is an almost exact replica of the actual bigram, both in respect of its time relations and in respect of the voltages represented by the several deflections. Note especially the notches marked by asterisks on the upstrokes of *R*. The calculated curve departs from the outline of the natural curve approximately some 0·0350 sec. after *R* begins, or approximately 0·0450 sec. after the first phase of the levogram. Ordinates are on the scale of 7·5 cm. = 1 millivolt. Abscissæ, 1 cm. = 0·02 sec.

There are two possible criticisms of these experiments. It may be argued that in clamping the right division certain branches of the left division are also injured. The reply to this criticism is to be found in protocols of experiments published later (p. 267); unless there is very extensive damage or complete transection of the left division the distribution to the left ventricle as gauged by the extracardial leads remains unaltered. The second criticism which may be raised is that in several applications of the clamp the main stem of the bundle is almost certain to suffer some damage. As a matter of fact, in one series of my curves 2 : 1 block was present; evidently in this circumstance the impulse was still of auricular origin, and the spread was therefore still through the usual Purkinje channels. I possess similar curves from this animal in which there was no block, but the curves, being tremulous, were less suitable for measurement, though of the same outline. Criticism applies chiefly to the other experiment in which complete heart-block was present (fig. 2, and Plate 18, fig. 12). The reply to this criticism is also that the curves are of the same form whether complete block is present or not. An experiment performed for the purpose is illustrated by Plate 18, figs. 13 and 14. In this animal a clean cut severed the right division of the bundle, and the usual curves were subsequently recorded (fig. 13); a clamp was now placed on the bundle stem and tightened until complete block was obtained (fig. 14). Despite the dissociation of auricular and ventricular rhythms, the form of ventricular complex remained practically unchanged. Such slight change as occurred is no more than is observed from time to time in an experiment in which the curves are repeatedly taken without further experimental interference. The uniformity of distribution to a given ventricle, when the contralateral bundle division is not conducting, before and after the production of auriculo-ventricular dissociation, is readily to be explained upon the usual assumption that in *A–V* dissociation the impulses spring from the bundle itself immediately below the seat of damage. As a matter of fact, full justification of the primary experiment lies in its end result: the algebraic relation between dextrogram, levogram and bigram. Such accurate fitting cannot be coincidental and can be explained only by the hypothesis suggested.

SEPARATE CONSIDERATION OF THE LEVOGRAM AND DEXTROGRAM.

We owe to EPPINGER and ROTHBERGER* the statement that lesions of one or other division of the *A–V* bundle produce profound modifications of the natural electrocardiogram in dogs. Their general statement and their account of the form of change has been accepted widely by a number of workers upon the physiology and pathology of the heart. I have accepted their general conclusions, and have had less hesitation in so doing because they have seemed to me to harmonise with a variety of physiological and clinical observations. At the same time, it should be stated that the report in question and the subsequent article of ROTHBERGER and

---

* 'Zeitschr. f. klin. Med.,' vol. 70, p. 1 (1910).

WINTERBERG* do not contain a sufficiently detailed statement of procedure and evidences to ensure, by themselves, unqualified support to the conclusions contained in them. EPPINGER and ROTHBERGER'S experiments have not as yet been confirmed by successful repetition.

As the conclusions of my present article must presume those of EPPINGER and ROTHBERGER'S to be valid, it has been necessary to seek corroboration of their observations. The general methods employed have been sufficiently recorded in previous articles. The curves have been studied in three leads from pairs of non-polarisable electrodes fixed to the right shoulder, left shoulder, and the mid-line of the abdomen, immediately above the umbilicus. A standardised curve has been taken from each lead (each simultaneously with a constant curve from a direct heart lead), before and after the insertion of the knife. Extra sets of curves have been taken whenever the change in the form of curves has been unstable. The extent of the lesion has been subsequently examined.

THE CHANGE IN THE ELECTROCARDIOGRAM PRODUCED BY LESIONS OF THE RIGHT BUNDLE DIVISION OF THE DOG.

Proof of the Association of the Lesion with a Particular Type of Electrocardiogram.

The right stem of the *A–V* bundle in the dog, after leaving the bifurcation, runs in the septum on its right side first in a ventral direction and in almost the whole of its course it is covered by endocardium and a connective tissue sheath only. In the majority of freshly opened hearts, its course can be faintly but distinctly traced with the naked eye. The branch curves, being convex toward the ventral surface of the heart, and finally enwraps the chief papillary muscle of the right ventricle. This course is displayed in the accompanying outline drawings (fig. 3).

These diagrams have been constructed accurately to scale, and with the septum displayed in a constant fashion; the free wall of the ventricle is detached by a cut running from the pulmonary orifice along the *A–V* groove, and finally ascending into the auricular wall; the freed ventricular wall (*F.W.*) is thrown downwards, and the attachments of those chordæ which go to the chief papillary muscle (*P*) are divided.

In the upper part of its course the bundle division (*B*) is hidden by the septal segment of the tricuspid valve (*S.T.*). When it reaches the papillary muscle it breaks up into a number of free and anastomosing branches. Other branches are frequently given off in the lower part of the convexity; and these may be visible (see fig. 3, *II*, *IK*); but more often they are found by microscopic examination. The dotted lines show that portion of the course of the bundle division which was visible in the fresh heart.

In my early experiments on the right bundle division I used a clamp, and, having lightly pressed with this instrument until the desired curves were obtained, increased the pressure and obtained a permanent effect. In a series of seven successful experiments on the dog, discoloration of the tissues or blood clot was found on the right side of septum, clearly overlying the right bundle stem; but it was also apparent in many of these experiments that some of the ventral branches of the left division were also involved. I cut three of the dog's hearts in serial sections. In one dog the region of crush could not be positively identified microscopically. In the other two the right division suffered chief damage, but the bifurcation of the bundle was also involved in spreading hæmorrhage.

---

* 'Zentralb. f. Herz- u. Gefässkrank.,' vol. 5, p. 206 (1913).

These experiments were regarded as indecisive, and the method of clamping was therefore abandoned.

In dividing the right division of the bundle I introduce a tenotomy knife through the centre of the conus muscle, immediately beneath the pulmonary valves; the blade is passed into the cavity on the flat and parallel to the *A–V* groove. Once through the wall, the point is carried a little towards the heart's apex to escape entanglement in the chordæ tendineæ, and the cutting edge is turned toward the septum. The knife is then pushed on until its point is actually in line with the base of the right auricular appendix, or a little beyond it. The cutting edge is applied to the septum, the cut made, and the knife withdrawn. Little or no hæmorrhage accompanies this operation, and a successful cut may be placed in at least 50 per cent. of experiments.

The figure illustrates eight experiments. Preliminary curves were always taken from the three leads, and extra sets during the course of the experiment. Each heart was examined by means of serial sections.

Dog I.J. (5·5 kgrm.). A cut was made at the ventral edge of and below the septal valve. The bundle division could be traced to the centre of it in the freshly opened heart and microscopically. The bundle was completely divided at the centre of the incision. Characteristic curves (fig. 17) at once developed, and these were maintained permanently.

Dog I.L. (5·0 kgrm.). Two cuts which fell together, and the first of which was ineffectual, were made. In this animal the course of the bundle division could be traced for a short distance only. It seemed to the eye to have been involved in the tails of the incision. The sections showed the bundle division to be completely transected by the upper of the two incisions at its extremity. Curves of small amplitude, but of characteristic form, developed at once and were maintained.

Dog I.M. (11·3 kgrm.). A single cut was made, into the edges of which the bundle division could be clearly traced. The sections showed transection of the bundle division. Characteristic curves at once developed and were permanently maintained.

Dog I.I. (9·3 kgrm.). The preliminary and physiological curves were of a somewhat unusual type. A cut was made below the septal valve. The bundle branch was readily traced into the middle of it, and sections showed it completely divided. The electrocardiograms at once changed. In Lead *II* the curve was of the usual and characteristic form; in Lead *I* it was of similar form and of equal extent; in Lead *III* it was anomalous.

Probably the atypical characters in Leads *I* and *III* were associated with an unusual distribution of the Purkinje tissues to the left ventricle; here the free strands were exceptionally numerous, and had an unusual arrangement; the physiological curves in this animal were equally anomalous. The change was permanently maintained.

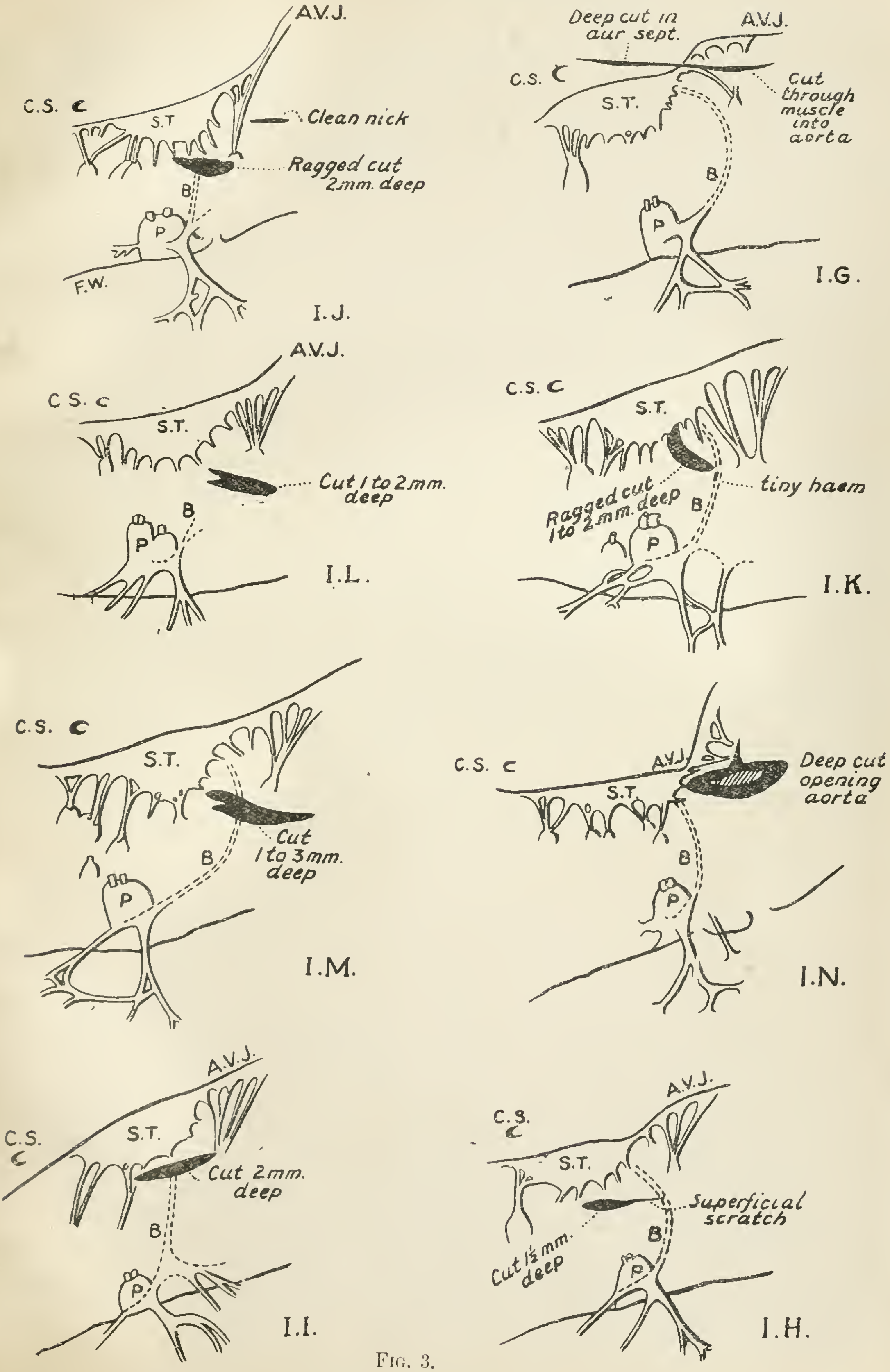

A.V.J.
C.S.
S.T
Clean nick
Ragged cut 2mm. deep
B
P
F.W.
I.J.
Deep cut in aur sept.
A.V.J.
C.S.
S.T.
Cut through muscle into aorta
B
P
I.G.
A.V.J.
C S.
S.T.
Cut 1 to 2mm. deep
B
P
I.L.
C.S.
S.T.
tiny haem
Ragged cut 1 to 2mm. deep
B
P
I.K.
C.S.
S.T.
Cut 1 to 3mm. deep
B
P
I.M.
C.S.
A.V.J.
S.T.
Deep cut opening aorta
B
P
I.N.
A.V.J.
C.S.
S.T.
Cut 2mm. deep
B
P
I.I.
A.V.J.
C.S.
S.T.
Superficial scratch
Cut 1½ mm. deep
B
P
I.H.

Fig. 3.

We pass to control observations.

Dog I.G. (8·0 kgrm.). A long cut was made at too high a level, and the sections showed the bundle and its branches to be untouched. It ran from a point above the coronary sinus (*C.S.*) ventrally, cutting the auricular septum deeply, crossing the *A–V* junction, and, penetrating the muscle of the ventricle over a length of 16 mm., opened the root of the aorta. A single curve was secured from Lead *II*; it had a natural outline. The heart dilated before the remaining curves could be photographed, but the curve of Lead *III* was seen to have remained unaltered.

Dog I.K. (11·1 kgrm.). A particularly instructive experiment. A cut was made in the customary situation. It was placed too obliquely. It had torn the edge of the valve and fell just short of the bundle division, as was clear macroscopically and microscopically. After a brief interval, during which the electrocardiogram remained unchanged, perfectly characteristic curves suddenly developed. They persisted for an hour, when the natural form of curve was again resumed. The course of the bundle division was marked opposite the end of the incision by a minute hæmorrhage, whose outlines were continuous with those of the bundle branch. The continuity of the bundle division was undisturbed, but at its ventral edge the lesion had distorted the bundle by crushing it a little and allowing blood to effuse around the fibres.

Dog I.N. (10·4 kgrm.). Cuts which fell together were made in the septum. The muscle was penetrated in the whole thickness immediately ventral to the septal valve segment. The course of the right bundle branch could be traced throughout; it was not involved. Curves taken from Leads *II* and *III* immediately afterwards were of normal outline, and differed but little from the preliminary curves. The curves of Lead *I* were not recorded, but it was seen that *S* had deepened somewhat; but by this time the right ventricle was rapidly dilating on account of the leak from the aorta.

Dog I.H. (5·3 kgrm.). A doubtful experiment. A small cut was made below the septal valve. As a muscular cut, it fell short of the bundle division, but was prolonged as a superficial scratch which penetrated the endocardium only. This scratch just crossed the branch, whose fibres were intact histologically. Characteristic curves were developed immediately. I did not continue the experiment, and the permanency or otherwise of the effect was not decided.

These observations seem to me sufficient to prove beyond reasonable doubt the relation between right bundle division lesions and the curves which are held to correspond to them in the dog.

The Curves Characterising a Lesion of the Right Division.

The electrocardiograms obtained when the right division of the *A–V* bundle is damaged are variable in form within certain limits. In the first place, I propose to describe in detail the commonest type (Plate 19, figs. 17 and 18), and, subsequently, to notice its variants. The broad features of the common type have been described by EPPINGER and ROTHBERGER.

In Lead *I* the auricular complex is followed by a ventricular complex of small amplitude, comprising preliminary and chief deflections. The preliminary deflections consist usually of a small downward, followed by a small upward, excursion; the former (*Q′*) has a value of 0 to 0·2 millivolt, the latter (*R′*) of 0·1 to 0·3 millivolt (Table 1). These preliminary deflections are succeeded by the first chief deflection, a downward excursion (*S′*) of from 0·2 to 1·3 millivolt; and the second chief deflection (*T′*) a rounded and prominent summit.

TABLE I.—Amplitudes of Deflections in Bigram and Corresponding Levogram.

Dog.	Bigram.									Levogram.									Method.
	Lead *I*.			Lead *II*.			Lead *III*.			Lead *I*.			Lead *II*.			Lead *III*.			
	Q.	R.	S.	Q.	R.	S.	Q.	R.	S.	Q′.	R′.	S′.	Q′.	R′.	S′.	Q′.	R′.	S′.	
H.V.	0	13	1	0	19	6	1	10	8	1	26	0	0	14	23	0	5	40	Clamp.
H.W.	1	13	0	0	23	2	tr.	9*	4	2	14	0	0	5	27	0	4	35	Clamp.
I.H.	0	10*	0	0	19	5	0	12*	8	0	5	2	0	8	16	0	7	18	Incision.
I.K.	tr.	11	1	0	20	4	tr.	7	12	tr.	6	1	tr.	10	23	1	7	25	Incision.
H.M.	0	7	0	0·5	21	3	0·5	15	6	0	5	0	1	11	29	1	9	32	Clamp.
H.P.	1	8*	0	2	17	4	1	12*	6	1	1·5	8	2·5	7	33	1·5	7	21	Clamp.
H.S.	0	3	2	tr.	5*	3	0	3*	1	0	1	3	tr.	3	26	0	2	25	Clamp.
H.T.	1·5	6*	0	1·5	3	5	1·5	4	10	1	1	6	0·5	6	24	tr.	4	19	Clamp.
I.J.	0	8	0	0	17	3	0	10*	4	0	3	6	0	6	23	0	3	18	Incision.
I.L.	0	5	0	0	13	0	0	7	0	0	2	2	0	6	5	0	3	3	Incision.
I.M.	0	4	0	0	8	1	0	5	0	0	3	6	0	3	19	0	2	11	Incision.
I.O.	—	—	—	—	—	—	—	—	—	1	1	13	tr.	8	23	0	9	15	Incision.

The amplitudes in this Table are expressed in tenths of a millivolt. Summits marked with an asterisk were double or notched. The chest was open while all the curves were taken.

In Lead *II*, two preliminary deflections are the rule; a small downward movement (Q') of value 0 to 0·25 millivolt is usual, and a summit (R') usually sharp, and of value 0·3 to 0·8 millivolt,[*] is constant. Then follow the chief deflections: S', a steep and prominent downward movement whose value usually falls between 2·0 and 3·0 millivolts, and the course of which is often broken by a little notch; T', invariably upwards and rounded, and of considerable amplitude.

Lead *III* gives an electrocardiogram which is very similar to that of Lead *II*, but its dimensions are less. Q' varies between 0 and 0·15 millivolt, and is not often present; R' varies between 0·2 and 0·9 millivolt; S', usually between 1·1 and 2·5 millivolts; T' has the same direction and almost the same prominence as in Lead *II*.

The general features of these curves which deserve stress are as follows:—

(1) The first chief deflection (S') is in the same direction, *i.e.*, downward, in all curves, and is usually most prominent in Lead *II*, a little less so in Lead *III*, while in Lead *I* it is diminutive by comparison. (2) The second chief deflection (T') is always opposite in direction to the first chief deflection. (3) The initial deflections (Q', R', S') occupy a considerable time interval, having a duration in Lead *II* of from 0·0708 to 0·1083 sec.; they comprise approximately one-third of the whole ventricular complex, and have almost twice the duration of the initial phases in the bigrams

---

[*] Occasionally the rise in the curve starts from a point so far below the base line that it fails to reach the latter.

of the same animals (see Table II). (4) The curves in Leads *II* and *III* have an amplitude which is much greater than those of the normal curves in the same animal.

TABLE II.—Time Lengths of Bigram and corresponding Levogram in Lead *II*.

Dog.	Bigram.			Levogram.			Weight.
	Heart rate.	Length of initial phases.	Length of whole complex.	Heart rate.	Length of initial phases.	Length of whole complex.	
							kgrm.
H.V.	120	0·0518	0·2678	94	0·1083	0·3754	7·1
H.W.	186	0·0426	0·1960	200	0·0986	0·2460	12·4
I.H.	125	0·0451	0·2826	117	0·0744	0·3098	5·3
I.K.	160	0·0443	0·2297	169	0·0901	0·2480	11·1
H.P.	174	0·0616	0·2280	174	0·0884	0·2458	35·0
H.S.	171	0·0459	0·2214	170	0·0888	0·2360	9·4
H.T.	169	0·0474	0·2280	145	0·0912	0·2817	13·0
I.J.	145	0·0431	0·2238	146	0·0773	0·2659	5·5
I.L.	120	0·0453	0·2420	109	0·0708	0·2958	5·0
I.M.	132	0·0401	0·2568	148	0·0887	0·2720	11·3
I.O.	—	—	—	132	0·0891	0·1796	8·4
H.M.	171	0·0502	0·2066	176	0·0807	0·2768	9·4
Average...	—	0·0470	0·2348	—	0·0872	0·2694	—

Now while these curves are discovered as a rule, important variations may be noted in Leads *I* and *III*. The usual type I shall term "concordant," because the chief phases are similar in direction in all leads. The less usual is the "discordant" type, when the chief phases are opposite in direction in Leads *I* and *III* (see Plate 19, fig. 16). In these it is the curve of Lead *I* which diverges most, the first chief phase (R') is upward, and may have an amplitude of 1·4 or 2·6 millivolt; it is followed by the second chief phase (T''), which is a downward deflection. When this type of curve prevails in Lead *I*, the curve of Lead *III* is not a diminished but an exaggerated picture of Lead *II*, and the amplitude of S' may reach 4·0 millivolts in Lead *III*.

Thus the general features of these curves are not identical with those already enumerated for those of the concordant type, though the two types resemble each other in many respects; the second and third numbered statements apply to the concordant and discordant curves equally, the fourth statement applies to the discordant type in all leads.

As we shall see in the sequel, the discordant or rarer type is identical with curves taken from the human subject, and ascribed by many writers to lesions of the right bundle division in man; and the most baffling section of the present work has been the search for the cause of these variations. At first, when I was in possession of

Eppinger and Rothberger's reports and of curves of my own of the concordant type, I believed that these alone occurred, and sought an explanation of their divergence from the "discordant" human curves. It seemed possible that the opening of the chest wall in the dog might materially affect them, but this was found not to be the case; the curves remain unchanged in their broad outlines when the chest wall is restored layer by layer and the lungs are fully expanded by extracting all air from the pleural sacs (see fig. 6). Next it seemed to me that the position of the heart might be responsible for the difference and, having ascertained the natural lie of the heart by studying published sections of the human chest and my own sections of the dog's chest, I carried out experiments in which the plane of my triangularly disposed contacts was altered on the restored chest wall, so that their positions relative to the heart might be as similar as possible in man and dog. In these experiments, also, I failed to find an explanation; but while they were in progress the first "discordant" curves were taken from a dog in which a straightforward experimental procedure had been employed. It then became evident that the reason lies in the heart itself, and the problem narrowed itself from an explanation of the different type in dog and man, to an explanation of two different types in the dog.

The variation in the type of electrocardiograms under discussion is unquestionably due to a variation in the original distribution of the excitation wave in the left ventricle. At one stage I expected that it might be due to differences in the relation of the lesion to the conducting system, so that in one instance the whole right stem suffered, while in another early outgoing branches escaped; but I failed to obtain any histological evidence for this view, and noticed further that in those hearts from which discordant curves had been taken the arrangement of the free Purkinje strands in the left ventricle was unusual; though numerous, they were much shorter than is customary and, lying closer to the wall, bridged the cavity to a less extent. But the most conclusive evidence was found in the natural bigrams, taken before the bundle division was damaged (Table I). In all instances where the discordant type of levogram was discovered, and where the first chief phase was upward (R') in Lead *I*, R in the bigram of the same lead was unusually prominent, amounting to as much as 1·3 millivolt (Dog H.V. and H.W.), whereas the average value of R in this lead is but 0·8 millivolt when the curves showing large values are included. Knowing that the bigram is of dual construction, and that the value of R in the bigram is partly dependent upon the value of R' in the levogram (a statement which evidently applies not only to Lead *II* where it has been fully demonstrated, but also to the remaining leads), we at once recognise why the discordant type of curve with its tall R' in Lead *I* is associated with an unusually tall summit R in the bigram of the same lead. But more important for our present purpose is the clear demonstration, which this association affords, that the discordant type is not the result of any variation in experimental procedure;

pointing, as it does clearly, to a pre-existing difference in the distribution of the excitation wave.

I may terminate this section by stating that in the dog concordant and discordant types are not sharply separated, but that intermediate types are encountered.

*The Meaning of Levograms and the Path of the Excitation Wave in the Left Ventricle.**

Discordant Curves.—For reasons which will be apparent subsequently, I propose to consider in the first instance those levograms which are of the discordant type.

In fig. 4 the initial phases of curves from Leads *I*, *II* and *III* have been plotted above each other. The method of constructing these charts has been explained in dealing with the toad's heart. In the dog I use as standard lead for orientation a direct lead from left ventricle to chest wall. The curves are all taken while respiration is suspended in the inspiratory position. I have plotted the curves for the first six hundredths of a second. Now these curves correspond to true levograms up to a point which is indeterminate, namely, to the last point where they represent spread in the left ventricle only. In writing of the dual construction of the bigram, it has been explained that, when the path to one ventricle is rendered functionless, the excitation wave flowing to the contralateral ventricle begins to spread from it to the homolateral ventricle after some 0·0350 to 0·0400 sec. or less subsequent to the inscription of the upstroke of *R*. The present charts are constructed so that the opening phase lies on the 0 line; consequently, the true

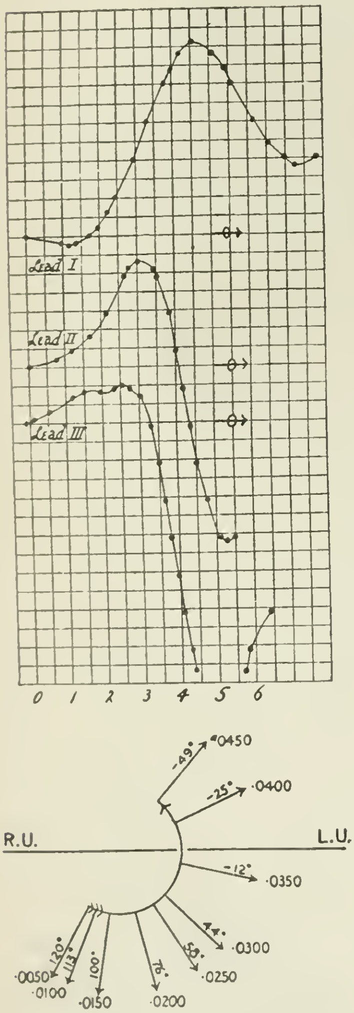


FIG. 4.—A chart showing the initial phases of the levogram, as it was recorded in Leads *I*, *II*, and *III* with the chest open (Dog H.V.). The curves are of the discordant type. The figures below the chart mark successive hundredth of a second lines. Ordinates, 1 cm. (four scale divisions) = 1 millivolt; abscissæ, 1 cm. (four scale divisions) = 0·02 sec.

Below is a diagram showing the electrical axes calculated for each 0·005 sec. The movement of the axis is uninterruptedly anticlockwise in its direction. The angles are expressed relative to the base line R.U.-L.U., which represents Lead *I*, from the right upper to the left upper limb of the animal. The data from which the angles were calculated are given in Table III.

---

* A preliminary report of these observations appeared in the 'Proceedings of the Physiological Society,' May 15, 1915.

levogram is represented for some 0·0450 to 0·0500 sec. or less, the interval between the beginnings of the curves and *R* being approximately 0·010 sec.

The values of these three charted curves are given in the accompanying Table at each 0·0050 sec. ; the curves fit, that is to say the values in Leads *I* and *III* are together equal to the values in Lead *II*. The corresponding angles, calculated trigonometrically, are recorded in the last column of the Table, and are drawn in the diagram beneath the chart (fig. 4). We see that the electrical axis is at first directed at an angle of 120° to the horizontal, and that it is rotated anticlockwise through 169°. The movement has not a uniform rapidity, being faster as the horizontal position is reached ; but this is a minor detail—the chief fact is the uninterrupted character of the rotation ; throughout it is in the stated direction. As in the case of the toad's heart, I judge this change in the direction of the electrical axis to express an alteration in the average direction in which the excitation wave moves. Interpreting the movement of the electrical axis in this wise, we may regard the diagram of rotation as expressing the change in the average direction of the excitation wave from instant to instant ; and may read in the early directions the passage of the excitation wave downward and to the right in the septum ; later its passage downward, or downward and to the left, at the apex of the ventricle ; later its passage to the left in the lateral wall ; and lastly its passage upwards and to the left in the lateral wall near the ventricular base.

TABLE III.—Dog H.V. (Levograms).

	I.	II.	III.	Angle.
sec.				
0·0050	– 1	1	2·5 2·0	120°
0·0100	– 2	3	5	113
0·0150	– 1·5	6·5	8	100
0·0200	2·5	11 10·5	8	76
0·0250	11	21	10	58
0·0300	21	28	7	44
0·0350	35	24	– 11	12
0·0400	44 45	4	– 41	– 25
0·0450	51	– 25	– 76*	– 49

The potential values in this and succeeding Tables, under columns I, II, and III, are expressed in 0·05 of a millivolt.

* Calculated ; Lead *III* off plate.

This interpretation agrees in a very precise fashion with the observations which ROTHSCHILD and myself have already published upon direct readings from the inner and outer surfaces of the ventricle, and with our general hypothesis that in the mammalian heart the excitation wave is distributed through the Purkinje system, and penetrates the ventricular musculature in a radiating fashion from the network. The curved arrow of the present diagram approximately represents the lie and direction of the Purkinje paths in a coronal plane.* The direction of the axis at given time phases corresponds in a very satisfactory manner with the actual time readings of endocardial and epicardial distribution of the excitatory process. We may choose from the readings given by ROTHSCHILD and myself, time readings which express in a broad fashion the distribution in the left ventricle. The readings for the lining of the left ventricle are a little uncertain, but we considered it probable that the excitatory process arrives in the cephalic end of the septum some hundredth of a second or more before the upstroke of *R*; while it reaches the apex of the ventricle at or about the time when *R* starts. On the outer surface the readings are affected by the thickness of the muscle; but, in general, they increase from apex to base, and increase to an extent which can be accounted for only by the disposition of the conducting tracts. The surface excitation wave appears at the base of the left ventricle at about 0·0250 sec. after *R*. Representative readings are utilised in the accompanying diagram (fig. 5); and to harmonise these with all the time phases of our chart (fig. 4) 0·0100 sec. has been added in each instance, being a fair allowance for the time interval between the beginning of *R* (to which the readings of ROTHSCHILD and myself were expressed) and the beginning of the levogram (which starts on the 0 line of our chart). My conception of the distribution would allow the excitation process to travel throughout the whole left ventricle in approximately 0·0350 sec.; commencing at 0 sec. in the cephalic end of the septum, it reaches the

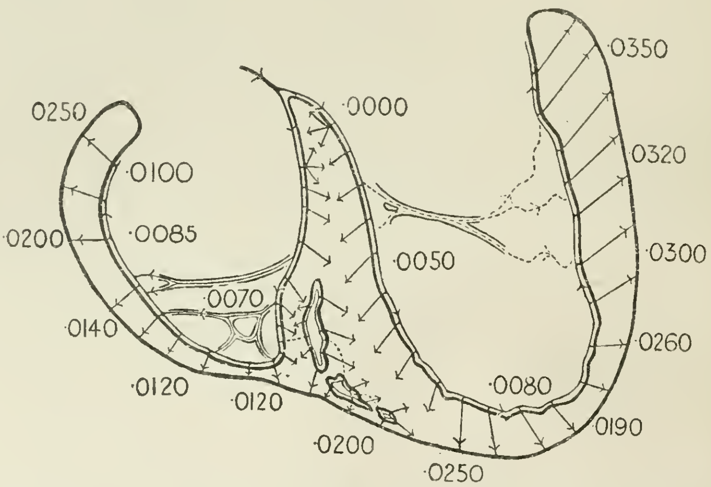


FIG. 5.—A natural size diagram of the dog's ventricle seen in section and illustrating the directions taken by the excitation wave in the spread through this heart chamber. The broken lines indicate spread through paths which do not lie in the actual plane of section. The figures illustrate direct readings in seconds from the epicardial and endocardial surfaces, and are related to the commencement of spread in the ventricle. The interval between this time instant and the beginning of *R* in the bigram may be taken at 0·0100 sec.

---

* More correctly in a plane inclined to the coronal; the question of this rotation will be discussed a little later.

surface of the lateral wall at the ventricular base at 0·0350 sec., passing in an anticlockwise fashion over septum to apex and later from apex to base over the free wall. Compare these readings with those of the movement of the axis in fig. 4 and a general agreement is observed, with the exception that the readings of fig. 4 are approximately 0·0100 sec. later than those of fig. 5 for corresponding time phases. This discrepancy is chiefly apparent near the end of the cycle, and is understood when we remember that the lining and surface readings represent the beginning of the rise of potential and not the full discharge. We read from the upstroke of the intrinsic deflection, not from its summit. The full discharge does not develop until at least 0·0100 sec. has elapsed from its commencement. There is, therefore, the nicest accord between surface readings and the direction of the electrical axis in the coronal plane; and this agreement is the strongest evidence of the correctness of my general hypothesis.

It becomes clear, if the hypothesis is sound, that the initial phases of the levogram in Lead *II** correspond to involvement of the septum in a left to right and base to apex direction, and that the summit *R′* corresponds to involvement of the apical musculature in a right to left and base to apex direction. The first chief phase (*S′*), on the other hand, represents involvement of the lateral wall, the direction in this structure altering gradually from a purely right to left to a right to left and apex to base direction.

Now *R′* of the levogram helps to build up *R* in the bigram, while *S′* is entirely responsible for *S* in the bigram.

Concordant Curves.—The movement of the electrical axis, corresponding to concordant curves, is not dissimilar to the movement just described. For the first 0·0250 sec. the movement in the two types is often almost identical. Thus in the example (fig. 6 and Table IV) the electrical axis starts at 150°, and moves in an uninterrupted anticlockwise fashion to −104°. In fig. 4 the movement up to 0·0200 sec. is to 76°; in the present instance it is to 77° (chest wall open). In fig. 4, at 0·0250 sec. the angle is 58°; in our present figure 30°. It is from this point onwards that the axis of concordant curves diverges from that of the discordant type. The axis moves rapidly across the horizontal 0° and takes up a position in the neighbourhood of −90°. In these concordant curves *S′* is almost equally prominent in Leads *II* and *III*, and it comes relatively early (at 0·0400 sec., as opposed to 0·500 sec. in fig. 4). The essential difference between the distribution in the concordant and discordant types is that in the former the large mass of muscle at the base of the left ventricle, the mass which is the last to be excited, is thrown into action earlier. I attribute this difference to the long free strands of Purkinje tissue which bridge the left ventricular cavity in these hearts (dotted paths in fig. 5). The bridging strands must inevitably affect the distribution of the excitation wave in the left ventricle, but their arrangement varies from heart to heart, and the actual variations which they engender are in all instances difficult to picture. But the feature of levograms which it is most important for us to recognise is the invariable tendency to an anticlockwise rotation of the corresponding electrical axis.

---

* Some levograms show the first spread to be horizontally to the right or even upward.

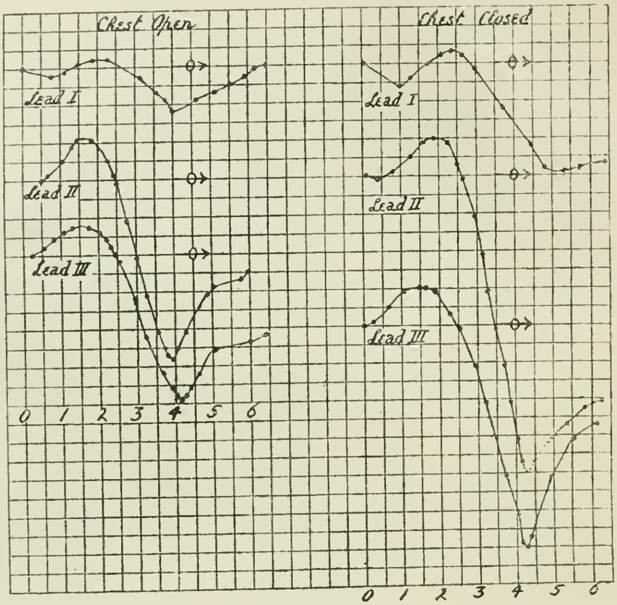


FIG. 6.—Charts showing the initial phases of the levogram in Dog H.T., and constructed in similar fashion to those of fig. 4. The curves are of the concordant type. The left-hand series was taken with the chest wall open; the right-hand series with the chest wall closed, and all air withdrawn from the pleural sacs through rubber tubes introduced for that purpose. In taking a single series of curves, the curve from each lead is taken simultaneously with a standard curve from a direct left ventricular lead. When the chest wall is to be closed, a long rubber tube filled with wool soaked in kaolin and passing through the chest wall, is employed as the ventricular contact. As is customary, the curves increase in amplitude, but are not materially altered in outline when the chest wall is closed. The angles formed by corresponding electrical axes are given in Table IV. Ordinates, 1 cm. (four scale divisions) = 1 millivolt; abscissæ, 1 cm. (four scale divisions) = 0·02 sec.

TABLE IV.—Dog H.T. Levograms.

	Chest open.				Chest closed.			
	I.	II.	III.	Angle.	I.	II.	III.	Angle.
sec. 0·0050	– 1·5	0	1·5	150°	– 3	0	3	150°
0·0100	– 1·5	4·5 4	5·5	103	– 6	3	9	131
0·0150	1	~~11~~ 9	8	84	– 2	8	10	101
0·0200	2	9 8·5	6·5	77	2	10	8	79
0·0250	1	0	– 1	? – 30	3	3	0	30
0·0300	– 2·5	– ~~18·5~~ 17·5	– 15	– 98	– 2	– 14	– 12	– 98
0·0350	– 7	– ~~38~~ 36	– 29	– 101	– 9	– 41	– 32	– 102
0·0400	– 12	– ~~48~~ 49	– 37	– 104	– 16	– ~~67~~ 66	– 50	– 104

In both the cited instances this rotation has been uninterrupted; in some instances there is not quite the same degree of uniformity in the direction of movement; there may be for short time intervals a little to and fro movement about a given angle (fig. 8 and Tables V and VII). Eventually, however, the axis resumes its accustomed revolution.

TABLE V.—Dog H.P. Levograms.

	Rotated.				Coronal.			
	I.	II.	III.	Angle.	I.	II.	III.	Angle.
sec.								
0·0100	−2	−5	−3	−113°	−2	−5	−3	−113°
0·0150	1	7	6	82	0	~~5~~ 4·5	4·5	90
0·0200	3	~~15~~ 16	13	80	3	13	10	77
0·0250	0	12	12	90	2	11	9	80
0·0300	−3	0	3	? 150	0	0	0	
0·0350	−3	−~~11~~ 10	−7	−107	−1	−16	−15	−94
0·0400	−2	−22	−20	−95	−5	−~~37~~ 36	−31	−97
0·0450	−5	−37	−32	−97	−9	−43	−34	−101
0·0500	−12	−~~55~~ 53	−41	−102	−15	−55	−40	−105
0·0550	−20	−~~64~~ 60	−~~39~~ 40	109	−22	−62	−40	−110

A final word completes this subject. I attempt to explain the rotation of the axis by assuming the passage of the excitation wave along the Purkinje paths of septum, apex and lateral wall in that order. As a matter of fact, however, the plane of our contacts has not the relation to the heart shown in fig. 5. Here the plane of section is at right angles to the septum; this plane in the dog makes with the coronal plane an angle of some 45–50°. In other words, the plane of the septum itself is not antero-posterior in the body, but is inclined to it, being approximately midway between the sagittal and coronal planes. Nevertheless, the electrocardiograms taken in the coronal plane clearly bear the impress of events in this mid-plane. If, while the chest wall is closed, we take two series of curves, the contacts being in the one instance in the coronal plane (Table V, coronal), and in the other instance being in a plane at right angles to the septum (Table V, rotated), the difference between the two series of curves is insignificant. It is clear therefore that the inclination of the plane of the septum does not vitiate the argument, or weigh materially against the general hypothesis. While some muscle elements excited along lines parallel to the plane of contacts will have, relatively, a preponderating influence upon the curves, while some excited in lines vertical to that plane will have, relatively, little influence, the main mass of muscle will be excited along lines of intermediate inclination; the electrical axis at a given instant will have a direction determined by complex factors, including the average direction of travel in all regions in process of activation and the masses of the muscular elements so involved. The

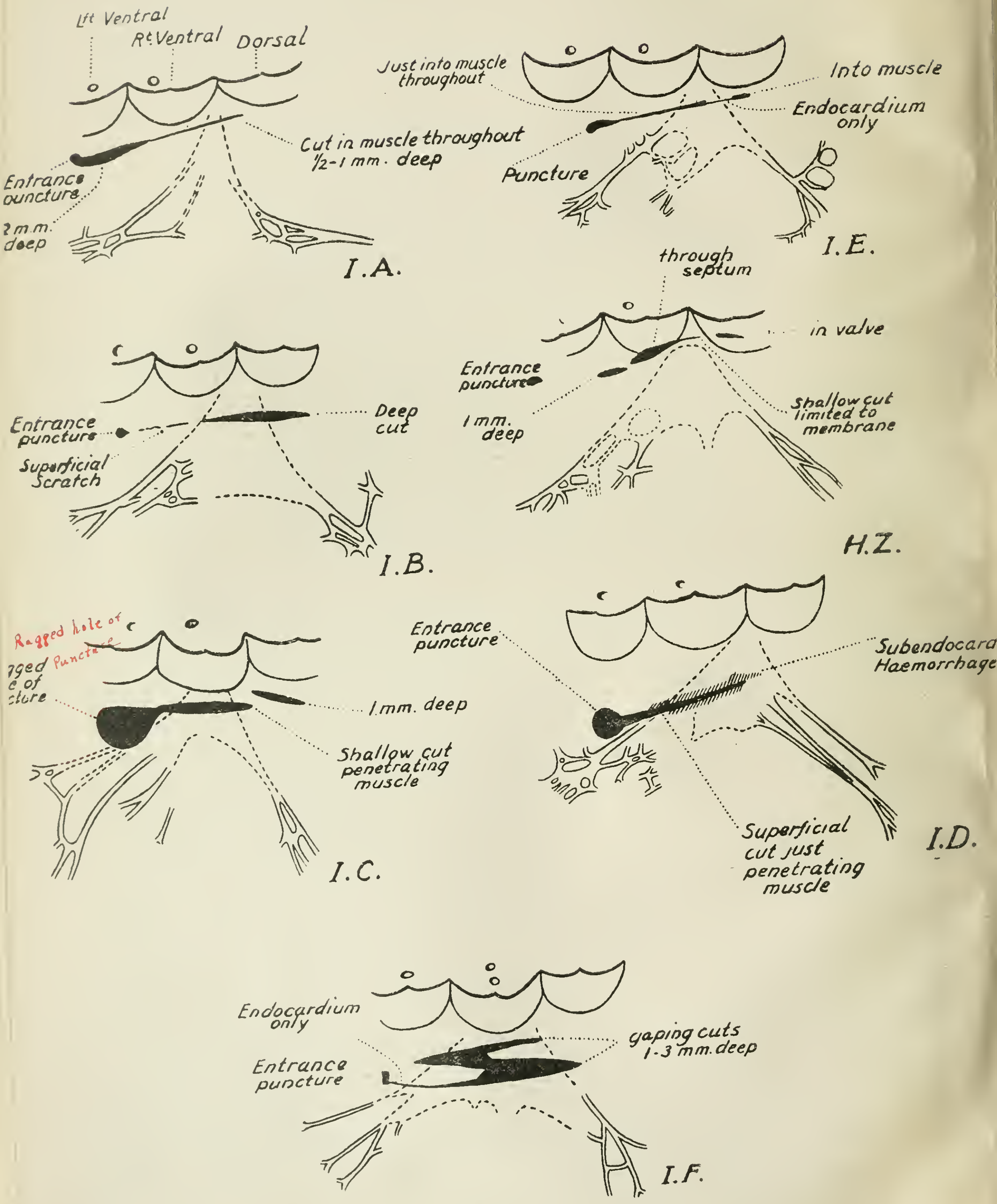

Lft Ventral
Rt Ventral
Dorsal
Cut in muscle throughout ½-1 mm. deep
Entrance ouncture
2 m.m. deep
I.A.
Just into muscle throughout
Into muscle
Endocardium only
Puncture
I.E.
through septum
in valve
Entrance puncture
1 mm. deep
Shallow cut limited to membrane
H.Z.
Entrance puncture
Deep cut
Superficial Scratch
I.B.
Ragged hole of Puncture
aged e of cture
1 mm. deep
Shallow cut penetrating muscle
I.C.
Entrance puncture
Subendocard Haemorrhage
Superficial cut just penetrating muscle
I.D.
Endocardium only
Entrance puncture
gaping cuts 1-3 mm. deep
I.F.

FIG. 7.

relation of the septum to the coronal plane is such that it will tend to emphasise the fanwise spread of the excitation wave to those parts of the free lateral wall which fall in the coronal plane.

The Change in the Electrocardiogram Produced by Lesions of the Left Bundle Division in Dogs.

Proof of the Association of the Lesion with a Particular Type of Electrocardiogram.

As is well known, the left division of the *A–V* bundle in the dog passes over and lies on the muscular septum and, appearing in an almost constant position a little way beneath the junction of two aortic cusps—the right ventral cusp (from which the right coronary artery springs) and the dorsal cusp (from which no artery arises)—it occupies a purely sub-endocardial position, and quickly spreads and flattens itself out to form a fan (see fig. 7), the outlines and many details of which are clearly visible in the freshly opened heart. The borders of this fan are continued into its two most prominent subdivisions,* the ventral and dorsal branches, which run a free course from septum to the bases of the corresponding papillary muscle. Usually originating as single free strands, they are subject to minor variations. Near their terminations in the sub-endocardial spaces overlying the papillary muscles they break up into a complex basketwork. These chief facts are illustrated in the accompanying diagrams, where free strands encased in endocardium are indicated by unbroken lines, and where the remaining visible outlines of the fan are represented by broken lines.

In cutting the left division I use a small tenotomy knife, introducing it, as did Eppinger and Rothberger, from the ventral and left surface of the left ventricle. The point chosen is the crutch formed by the descending branch of the left coronary artery at its union with its chief branch, an artery which runs down toward the left and dorsal surfaces of the heart's apex. The knife is driven cleanly in between the two arteries, in a dorsal direction, with an inclination to the right; it enters the cavity $\frac{1}{2}$–1 cm. below the left anterior aortic cusp (see fig. 7), and in the space between the mitral valve and septum. The point is carried back until the dorsal border of the system is reached, when the cutting surface is opposed to the septum. A light pressure is exerted and is maintained, the knife being withdrawn *during the progress of the next systole* into the hole of entrance.

The figure illustrates seven experiments. In two preliminary experiments in which another route was adopted, several short and ugly lacerations of the septum, evidently failing to transect the bundle division, were without appreciable influence upon the electrocardiogram. The illustrations have been constructed from the freshly excised specimens and are accurately to scale. They may be described in the order in which they are figured. The description of the lesions is based upon the fresh appearance and subsequent histological examination in each instance.

---

* The remaining subdivisions course down the septum.

Dog I.A. (10·1 kgrm.). A single cut was made. The lesion included the entrance puncture, a wound 1 cm. in length and 2 mm. deep ventral to the bundle division, a shallow cut of 2 cm. extent and varying from ½ to 1 mm. in depth, which ran upwards and included the whole of the fan at its base and transected it, as serial sections showed, completely. These wounds were all continuous in one line. The muscle was reached and cut along the whole line.

As a result the electrocardiogram immediately changed, the changes being perfectly characteristic in type, and being maintained permanently.

Dog I.B. (7·35 kgrm.). The lesion extended along a line of 3·4 cm. extent, and consisted of a small and isolated puncture, a superficial scratch ventral to the conducting strand and a deep clean incision of 1·8 cm. extent traversing the base of the fan completely. Macroscopically, the ventral extremity of the incision alone gave rise to doubt; the microscopic examination showed that the edge of the fan had been overlapped by the incision to a greater extent than is shown in the diagram.

Characteristic curves immediately developed and were maintained permanently.

Dog I.C. (15·8 kgrm.). The hole of puncture was long and ragged, involving the ventral border of the fan; running into it was a cut, appearing to penetrate the muscle along its whole length, crossing the base of the fan and dividing cleanly all but a few fibres at the dorsal margin; these showed evidence of having been crushed, and were densely infiltrated with extravasated blood corpuscles.

Characteristic curves (the initial phases of which are charted in fig. 9) immediately developed and were maintained.

Dog I.F. (8·1 kgrm.). Characteristic curves developed, and these persisted. The lesion was extensive, consisting of the hole of puncture and gaping cuts arranged in the fashion of a Z, which everywhere reached muscle. The bundle division was completely transected.

In the remaining experiments the incisions were placed less accurately, but these experiments are valuable as having supplied admirable controls.

Dog I.E. (7·6 kgrm.). The knife was inserted and withdrawn; the curves taken before and after the incision were of natural form, the two sets varying from each other in small details only. While the second set was being completed, characteristic curves of a left bundle lesion appeared. As soon as these were photographed, the natural form of electrocardiogram was resumed and persisted throughout the remainder of the experiment.

The lesion consisted of the hole of puncture continued into a shallow cut penetrating the muscle. It crossed the ventral border of the fan and cut the bundle division through two-thirds of its breadth. A short incision of similar depth was found in line with it, past the dorsal border of the fan, the two incisions being joined by an extremely shallow cut penetrating the endocardium only. The continuity of the bundle was intact over a considerable area.

Dog II.Z. (13·9 kgrm.). The broken cut was placed a little too high. In serial sections the cut was found immediately above the bundle bifurcation and the origin of the left division; its distance from the bundle varied from ½ to 1 mm. in most places; at one point the incision came nearer to the division of the bundle, slicing off a very small piece of the tissue from the upper margin of the saddle. Some blood had effused into the tissues overlying the bundle division and the left branch, and at one point seemed to have exerted pressure on these structures. The continuity of the majority of the bundle fibres, and the complete continuity of the left bundle division was clearly established, although the lesion was extensive, the tissues of the septum being penetrated at some places completely, at others almost completely.

The curves taken before and after the incisions presented no material differences in form, being natural. Large excursions of the string seemingly characteristic of a left branch defect were seen but not recorded from Lead *II* for a few seconds immediately after the incision.

Dog I.D. (9·4 kgrm.). For a short while after the incision had been made the natural curve in Lead *II* was maintained; then there was an abrupt change to the characteristic curves of a left bundle lesion. These persisted sufficiently to permit of their being recorded, when a more natural type was once more resumed and persisted.

The cut crossed the ventral border of the fan and divided two-thirds of its breadth, leaving the dorsal border uninjured. An area of subendocardial hæmorrhage, the extent of which is indicated by the shaded area of the diagram, surrounded the incision and extended dorsally for 2 mm., almost but not quite completing the transection.

The observations recorded, including as they do a very fortunate series of controls, seem to me sufficient to prove beyond question the relation between lesions of the left bundle division and the curves which are held to correspond to them in the dog.

The Curves Characterising a Lesion of the Left Division.

The curves characterising lesions of the left division (Plate 20, fig. 20) are variable in certain respects.

In Lead *I* the auricular complex is followed by a ventricular complex which is, in its broad outline, diphasic. There are, as a rule, no preliminary phases in any of the three leads. The first phase is directed upward, the second is directed downward* and is blunt (*T′*); the first phase, which is the more prominent of the two, is often divided; its amplitude varies between 0·8 and 2·2 millivolts (Table VI).

In Lead *II* the curve is also broadly diphasic, the first phase (*R′*) being almost invariably divided at its summit. It has an amplitude varying between 2·5 and 5·0 millivolts. The second phase (*T′*) is very prominent, and has an invariable downward direction.

In Lead *III* the curve is of the same form but smaller. The first phase, frequently divided at its summit, has an amplitude varying between 1·2 and 3·3 millivolts. *T′* is a prominent downward deflection.

The general features of these curves which are to be emphasised are as follows:—

1. The first chief deflection (*R′*) is in the same direction, *i.e.* upward, in all leads, and is always of greatest prominence in Lead *II*; it is usually less prominent in Lead *I* than Lead *III*, but the amplitude may be equal in these leads, or the relation may be reversed exceptionally.

2. The second chief deflection (*T′*) is always downward, that is to say, of opposite sign to the first phase, with isolated exceptions in Lead *I*.

3. The initial deflections (usually consisting of *R′* alone) are long, having in Lead *II* a duration of from 0·0824 to 0·1133 sec.; they comprise approximately one-third of the whole ventricular complex, and have almost twice the duration of the initial phases in the bigrams of the same animals (see Table VII).

4. With rare exceptions the curves in Leads *II* and *III* have amplitudes considerably surpassing those of the corresponding bigrams.

---

* Exceptionally, and when the first phase is diminutive, the second phase may have an upward direction.

Exceptionally, preliminary phases are also encountered; these may take the form of minute depressions or more rarely distinct depressions or distinct elevations in Leads *I* and *II*. In Table VI preliminary depressions have been expressed in the columns *Q′*. As in the case of the levograms, I attribute the more prominent variations to differences in the distribution of the Purkinje substance.

Table VI.—Amplitude of Deflections in Bigram and corresponding Dextrogram.

Dog.	Bigram.									Dextrogram.					
	Lead *I*.			Lead *II*.			Lead *III*.			Lead *I*.		Lead *II*.		Lead *III*.	
	Q.	R.	S.	Q.	R.	S.	Q.	R.	S.	Q′.	R′.	Q′.	R′.	Q′.	R′.
H.R.	0	9	0	0	35	0	2	16	0	0	8	0	25	0	24
I.A.	3	18	0	2	23	3	0	14	5	4	22	5	42	1	22
I.B.	tr.	4	1	0	29	3	0	27	3	0	18	0	50	0	33
I.C.	3	17	0	2	23	0	tr.	14	5	0	9	0	25	0	17
I.D.	1	20	0	0	23	5	0	13	9	1	22	0	45	0	28
I.E.	0	18	1	0	15	11	0	10	16	0	12	0*	37	0	33
I.F.	—	—	—	—	—	—	—	—	—	0	13	0	25	0	12

The amplitudes are expressed in tenths of a millivolt.

* In this animal a preliminary spike was shown in Lead *II*.

Table VII.—Time Lengths of Bigram and corresponding Dextrogram in Lead *II*.

Dog.	Bigram.			Dextrogram.			Weight.
	Heart rate.	Length of initial phases.	Length of whole complex.	Heart rate.	Length of initial phases.	Length of whole complex.	
							kgrm.
H.R.	186	0·0477	0·2046	190	0·0846	0·2018	16·2
I.A.	115	0·0538	0·2836	96	0·1011	0·2750	10·1
I.B.	125	0·0466	0·2846	83	0·1133	0·2970	7·3
I.C.	200	0·0444	0·1948	132	0·0824	0·2621	15·8
I.D.	114	0·0500	0·3080	120	0·0918	0·3152	9·4
I.E.	68	0·0590	0·3338	58	0·1030	0·3658	7·6
I.F.	—	—	—	136	0·0890	0·2874	8·1
Average .	—	0·0502	0·2682	—	0·0950	0·2864	—

The Meaning of the Dextrogram and the Path Taken by the Excitation Wave in the Right Ventricle.

The initial phases of the dextrogram have been plotted from a number of experiments. These curves represent true dextrograms up to a time phase lying some 0·0350 to 0·0400 sec. after *R*, that is to say, up to 0·0350 to 0·0400 sec. after the beginning of the *R′* in the dextrogram. Up to this point they may be utilised in calculating the direction of the natural electrical axis of the right ventricle. The common direction of the electrical axis of the right heart and its customary movements are represented most clearly in the first series plotted in fig. 9. Beginning at an angle of 30° there is a steady clockwise movement until the time 0·0250 sec. is reached; the angle of 70° or thereabouts is subsequently maintained. A clockwise movement at the first is the rule; the axis eventually sets between 50° and 90° in different experiments. Exceptionally the axis is almost steady between 70° and 90° from first to last; and in an isolated instance there was a slight anticlockwise movement.

The first direction of the axis is, as a rule, to the left and downwards; I interpret this to be the result of the response of the right side of the septum to impulses received from the right division of the bundle. Certain curves appear to indicate a preliminary upward movement in the septum, a phenomenon also witnessed at times in the left ventricle. The movement of the axis in a clockwise fashion I ascribe to gradual involvement of the lateral wall. The set of the axis to an angle approaching 70° seems to be accounted for by the general and simultaneous involvement of the free lateral wall and the trabeculated region. *A priori* it might be anticipated that a uniform clockwise movement of the axis would be found for the right ventricle, the excitatory process spreading down the septum and, later, gradually up the lateral wall. But this is not strictly the case. The architecture of right and left ventricles, and the distribution of the Purkinje arborisation to these two chambers, is different. We may exclude consideration of the conus, for in this structure the excitation wave progresses in planes almost at right angles to the plane of our contacts, and evidently can produce little effect upon the dextrogram; of the remainder of the right ventricular muscle it cannot be said that the basal muscle preponderates materially, as is the case in the left ventricle; for the apex of the right ventricle is blunt and consists of an extensive and heavy spongework, the trabeculated region. Our surface readings show us that the base of the left ventricle (in such a plane as is diagrammatised in fig. 5) receives the excitation wave little if at all earlier than the extreme apex of the right ventricle; when the axis sets it appears to express the component of the electrical forces which are set free by the almost simultaneous activities of these two muscle masses. The general direction in which the trabeculated region is activated, as judged by anatomical considerations, is strongly downwards and to the left; the general direction at the right base, as judged anatomically and experimentally, is to the right and somewhat upward. The resultant electrical axis is downward. The general activation of the right ventricle in the dog in a downward direction results from the complete and extensive bridging of the cavity by free strands of Purkinje tissue (see fig. 5). Taking account simply of the free wall of the right ventricle, the uppermost free strands join this wall at or a little below its centre point; but if we consider the whole of the right ventricle, including the trabeculated region, which is also supplied by the right bundle division, then the uppermost free Purkinje strand approaches the base of the ventricle much more closely than the apex. A general movement of the excitation wave in a base-apex direction is therefore to be anticipated in the dog. The clockwise movement which obtains in the heart of man (to be described) is masked or destroyed in the dog by what may be spoken of as a short-circuiting of the apical regions of this ventricle, brought about by the conspicuous and numerous bridges traversing the right ventricular cavity in this animal.

In the axial dextrogram the curve *R* rises at first gradually, and subsequently with increased steepness. The first part of the rise is attributable to septal activity, later the rise is enhanced when the trabeculated region and the lower part of the free wall becomes involved. Selected readings from the paper by ROTHSCHILD and myself accord with the view here expressed (fig. 5). Adjusting our readings* to the present charts and diagrams, the first surface reading comes at about 0·0120 sec., the earliest full discharge on the surface† comes certainly as late as 0·0220 sec.

At this time phase, when the electrical axis is beginning to set, the surface discharge is developing fully in the "central region," namely, the region where the free wall is attached to septum. From this time onwards, till the full development of discharge at the base (0·0350–0·0400 sec.) and at the extreme apex (0·0350–0·0400 sec. or a little less), the direction of the axis remains almost constant.

THE INTERPRETATION OF THE INITIAL PHASES OF THE DOG'S BIGRAM.

We are now in a position to complete our survey of the initial phases of the natural electrocardiogram. The chief conclusions have already been foreshadowed. The natural electrocardiogram is of dual construction, consisting of a summation of levogram and dextrogram. This has been shown for Lead *II*, and its demonstration for the remaining leads is, strictly speaking, unnecessary. But if further proof of the conjoint character of the natural curve were desired, it would be found in such charts as are published in figs. 8 and 9. In these charts the initial phases of the bigram have also been plotted, the time phases of bigram and levogram (fig. 9) or of bigram and dextrogram (fig. 9) corresponding accurately.* Possessing two of the three sets of curves, we may calculate the third; thus, if bigram and dextrogram are plotted from observation, the levogram can be ascertained algebraically (fig. 9). *The calculated levogram* (fig. 9) *and the calculated dextrogram* (fig. 8) *possess their usual outlines and magnitudes, and the directions of the electrical axes and their movements correspond closely with examples actually observed.*

---

* Allowing 0·0100 sec. for the interval between the commencement of the excitation wave in the ventricle and the beginning of *R*.

† Judged by the distance from the beginning to the summit of the intrinsic deflection.

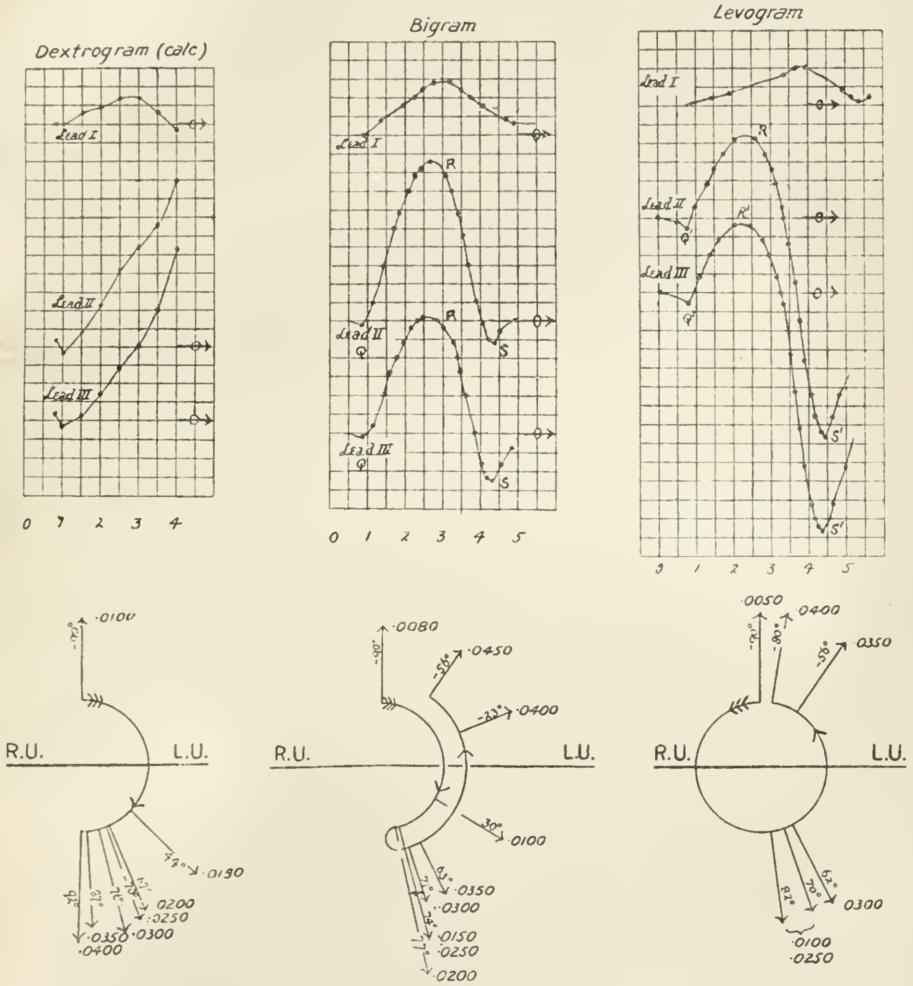


FIG. 8.—Three series of curves with corresponding diagrams of the electrical axes and their rotations (Dog H.M.). The left-hand series is of the calculated dextrograms, the central series of the actual bigrams, the right-hand series is of the actual levograms. The angles formed by the electrical axes are given in Table VIII. Ordinates, 1 cm. = 1 millivolt; abscissæ, 1 cm. = 0·02 sec.

These complete charts are particularly serviceable in that they demonstrate the composition of the bigram in the clearest fashion. In fig. 8 the axis corresponding to the bigram moves at first in a clockwise, and later in an anticlockwise, fashion.

---

* The plotting against time is accomplished by maintaining a standard lead from the left ventricle while bigram and levogram are photographed, or from the right ventricle while bigram and dextrogram are photographed.

Table VIII.—Dog H.M.

	Levogram.				Bigram.				Calculated dextrogram.			
	I.	II.	III.	Angle.	I.	II.	III.	Angle.	I.	II.	III.	Angle.
sec. 0·0050	0	– 1	– 1	– 90°								
0·0080	—	—	—	—	0	– 1	– 1	– 90°				
0·0100	1	3	2	70	1	1	0	? 30	0	– 2	– 2	– 90°
0·0150	2	~~13~~ 14	12	82	5	18	13	74	3	4	1	44
0·0200	3·5	~~21~~ 21·5	18	81	8	33	25	77	4·5	11·5	7	67
0·0250	5	~~21~~ 22	17	77	12	~~42~~ 43	31	74	7	21	14	70
0·0300	7	~~14~~ 15	8	62	14	~~41~~ 42	28	71	7	27	20	76
0·0350	9	– 7	– 16	– 56	12	26	14	63	3	33	30	87
0·0400	9·5	– 44	– 53·5	– 80	8	1	– 7	– 23	– 1·5	45	46·5	92
0·0450	7	– 57	– ~~61~~ 64	– 84	5	– 4	– 9	– 56	– 2	53	55	88

The first, or clockwise movement, which happens while the upstroke of *R* is inscribed, is clearly governed by the dextrogram. The turning point, where clockwise gives place to anticlockwise movement, corresponds to the summit of *R*; it is at this instant that *S'* in the levogram begins, and from this point onwards the levogram governs the bigram, and the anticlockwise movement is maintained. In the second example (fig. 9 and Table IX) the bigram opens with an angle of –110°, evidently governed by the levogram; for the next 0·0300 sec. it is governed chiefly by the dextrogram; at its termination the levogram once more predominates.

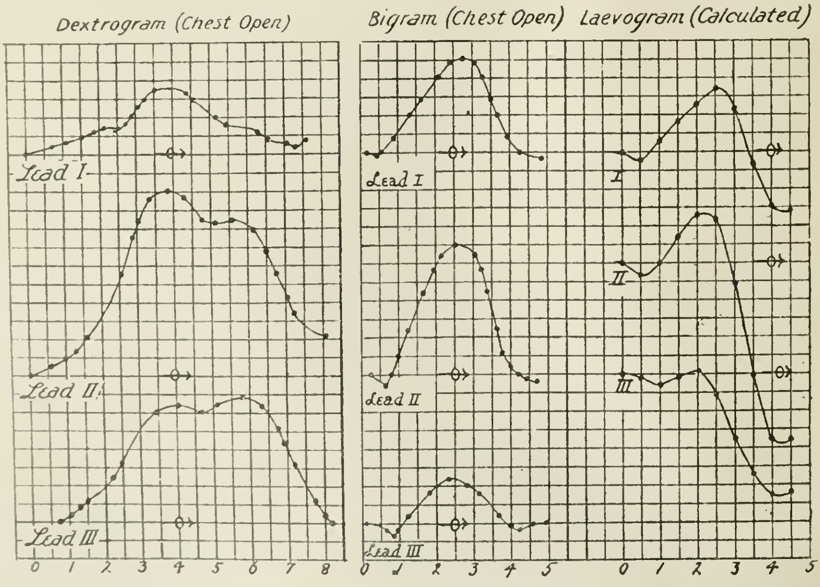


Fig. 9.—Four series of curves from Dog I.C. The left-hand series shows the initial phases of the actual dextrogram with chest open. The second series is that of the actual bigram. The third series is that of the levogram, calculated from the curves of dextrogram and bigram. The data from which the electrical axes were calculated are given in Table IX. Ordinates, 1 cm. = 1 millivolt; abscissæ, 1 cm. = 0·02 sec.

TABLE IX.—Dog I.C.

	Dextrogram. Chest open.				Dextrogram. Chest closed.				Bigram. Chest open.				Levogram (calculated). Chest open.			
	I.	II.	III.	Angle.	I.	II.	III.	Angle.	I.	II.	III.	Angle.	I.	II.	III.	Angle.
sec. 0·0050	2	2	0	30°	1	2·5	1·5	67°	−0·5	−1·5	−1	−110°	−2·5	−3·5	−1	−134°
0·0100	3	5	2	53	0	~~3~~ 4	4	90	6	5	−1	21	3	0	−3	? −30
0·0150	4	10	6	66	−1	9	10	95	12	~~18~~ 17	5	47	8	7	−1	23
0·0200	6·5	16	9·5	66	1	18	17	87	19	29	10	50	12·5	13	0·5	32
0·0250	7	~~26~~ 24·5	17·5	74	11	~~32·5~~ 32	21	70	24	~~35~~ 36	12	49	17	11·5	−5·5	12
0·0300	12·5	~~40~~ 39	26·5	71	23	51	28	63	24	33	9	45	11·5	−6	−17·5	−50
0·0350	17·5	~~49~~ 48·5	31	69	27	72	45	68	14	18	4	42	−3·5	−30·5	−27	−96
0·0400	18	50	32	69	28	83	55	71	3	2	−1	10	−15	−48	−33	−108
0·0450	15	46	31	71	27	82	55	71	−1	−2	−1	−120	−16	−48	−32	−109

In dealing with the toad and tortoise heart I have shown that the initial phases (R and may be S) cover that period of the heart's cycle during which the muscle mass, as a whole, is passing into the state of excitation. In the case of the dog's heart, I come to a similar conclusion; and it is based primarily upon the correspondence between the periods of the initial phases of levogram and dextrogram, respectively, and the periods covering readings from the surface and lining of the corresponding ventricles. But this statement is true only if we consider those phases which are parts of the true levogram and true dextrogram; that is to say, if the downstroke of R' in the dextrogram and a good deal of its plateau, and if the upstroke of S' in the levogram are not included. Treating the time-relations in a broad way, it is more satisfactory to deal with the bigram itself. The initial phases of this curve, stretching from the beginning of Q to the return of S to the base line, have an average duration of some 0·0480 sec. (see Tables II and VII). The readings of intrinsic deflections lie between 0·0000 and 0·0350 sec.; they show us that the whole ventricular muscle is activated within 0·0350 sec.; but the full discharge is not developed at the latest points to be activated until some 0·0100 sec. has elapsed. Thus the stage of complete activation, as estimated from direct readings, is approximately 0·0450 sec., a figure in close agreement with the length of the initial phases of the axial lead. *Thus the length of the initial phases of the bigram may be taken to indicate the duration of the process of activation.*

The initial phases of the levogram and of the dextrogram are of longer duration, and this is evidently due to the abnormality and late spread in right or left ventricle. When the right or left bundle division is cut, we may judge from the lengths of the initial phases before and after section, that the process of activation is retarded by some 0·0400 sec. (see Tables II and VII). For the initial phases of the levogram or dextrogram exceed those of corresponding bigrams by some 0·0400 sec. In strict accord with this estimate is the alteration in the values of readings over the right ventricle when the right bundle division is divided; they rise by approximately 0·0400 sec. (see figs. 12 and 13 of the paper by ROTHSCHILD and myself, and fig. 1 of the present monograph). There is a similar rise in the value of readings over the left ventricle, when the left bundle division is divided. Where does this delay occur? The main delay is not due to an altered direction of spread in the affected ventricle, though this factor is almost certainly in some small measure responsible; but it is conditioned by slow conduction across the septum, the path which the excitation wave must necessarily take in passing from one ventricle to the other when a bundle division is divided. This is evidenced not only by the almost uniform delay of the readings over the surface of the affected chamber, but by our direct observation of slow conduction across the septum (see previous communication). The plateau and fall of R' in the dextrogram and the slow rise of S' in the levogram are simultaneous with the abnormal and delayed spread in the left and right ventricle respectively. The close correspondence between the time period covering a complete series of direct readings and the time period occupied by the initial phases, in a variety of animals and in hearts excited along different paths, contributes the weightiest evidence that our direct readings are reliable.

Finally, I may sum up* the origin of the initial deflections of the dog's electrocardiogram in conclusions which apply to all leads :—

1. *Q, the first deflection of many electrocardiograms, is due in most dogs to activation of the septum from the left bundle division;* the chief evidences for this statement are :—(*a*) that the earliest reading of an intrinsic deflection has been obtained from the septum beneath the aortic valves; (*b*) that the corresponding electrical axis has a usual direction from left to right† ; (*c*) that Q is almost always constituted by the levogram‡ ; (*d*) that Q is absent in axial electrocardiograms from the toad and tortoise, in which animals no septum exists.

To this origin of Q I attribute its relatively frequent appearance in Lead *I* in the dog.

2. *R is in greater part a right, and in lesser part a left, ventricular effect and is mainly due to the progress of the excitation wave down the septum and its adjoining structures.* This conclusion is based :—(*a*) upon the interpretation of R' in the levogram and dextrogram respectively : in the levogram R' represents an average movement of the excitation wave downward to the right, in the dextrogram a movement downward and to the left ; (*b*) upon the observation that the upstroke of R is written before any surface reading is obtained, is at its height when the surface potential is fully developed over the thinnest region of the right ventricle in the central region of the heart ; and (*c*) upon its consistency with such septal readings as have been obtained.

3. A division of R, or notching of R, is explained by lack of synchronism between the summit of R' in the levogram, and the second or main turning point ; the latter is due to a balance established between the ascending limb of R' in the dextrogram and the descending limb of S' in the levogram. These conclusions are based upon an analysis of the composite parts of bigrams.

4. *S is known to result from an upward movement of the excitation wave through the basal and lateral wall of the left ventricle,* because :—(*a*) S is always contributed solely by the levogram ; (*b*) it corresponds to an outwardly and upwardly directed electrical axis ; (*c*) the conclusion is consistent with surface readings from this part of the ventricle.

---

* A preliminary summary of this part of the monograph was published in the 'Proceedings of the Physiological Society,' July 3, 1915.

† In some instances the direction is almost vertically upwards ; the exact manner of Q's production in these circumstances is somewhat uncertain.

‡ In some few instances the dextrogram apparently contributes in small measure.

APPENDIX.

The following illustrative experiment, while forming no essential part of the foregoing observations, has many interesting and indirect relations with them.

The ventral surface of a dog's heart being exposed (fig. 10), the muscle was excited at a number of points (1–14), using at each point successive threshold stimuli. The corresponding responses of the heart were recorded in Lead *II*, and single ventricular cycles are shown to the left in the figure. The signal of stimulation was also recorded (see Curve 13), and in each instance the time interval between the signal of stimulation and the first prominent deflection was measured. These intervals are tabulated (Table X). Subsequently the heart was hardened in a natural condition of diastole, and an oblique section was cut passing through the points of stimulation along the line 3–13. A diagram of this section is also represented in the same figure. The distance of the ventricular surface to the Purkinje network was measured in millimetres at all stimulated points.

TABLE X.

Point stimulated.	Distance to Purkinje system.	Signal to chief phase.
	mm.	sec.
1	R. 4	0·0363
2	R. 3	0·0416
3	R. 3	0·0424
4	R. 4	0·0405
5	R. 3	0·0407
6	R. 3·5	0·0473
7	R. 1·5	0·0293
8	R. 2	0·0245
9	R. 2	0·0343
10	R. 3	0·0497
11	R. 7 L. 8	0·0686
12	R. 12 L. 10	0·0920
13	L. 9·5	0·0920
14	L. 8	0·0699

If the series of curves is examined, it will be noticed that the ventricular responses obtained over the whole ventral surface of the right ventricle yield very similar outlines in the electrocardiogram; these vary from each other in detail and magnitude.* As the point of stimulation is moved along the *A–V* groove from the right towards the left margin and on to the conus, the excursion of the string, in its response to the heart beat, increases somewhat in magnitude. As the stimulating

---

* The curves are complicated by the presence of auricular complexes; these are of the type accompanying natural auricular beats in the case of Curves 3, 4, and 5; the remaining curves show auricular complexes of the retrograde type.

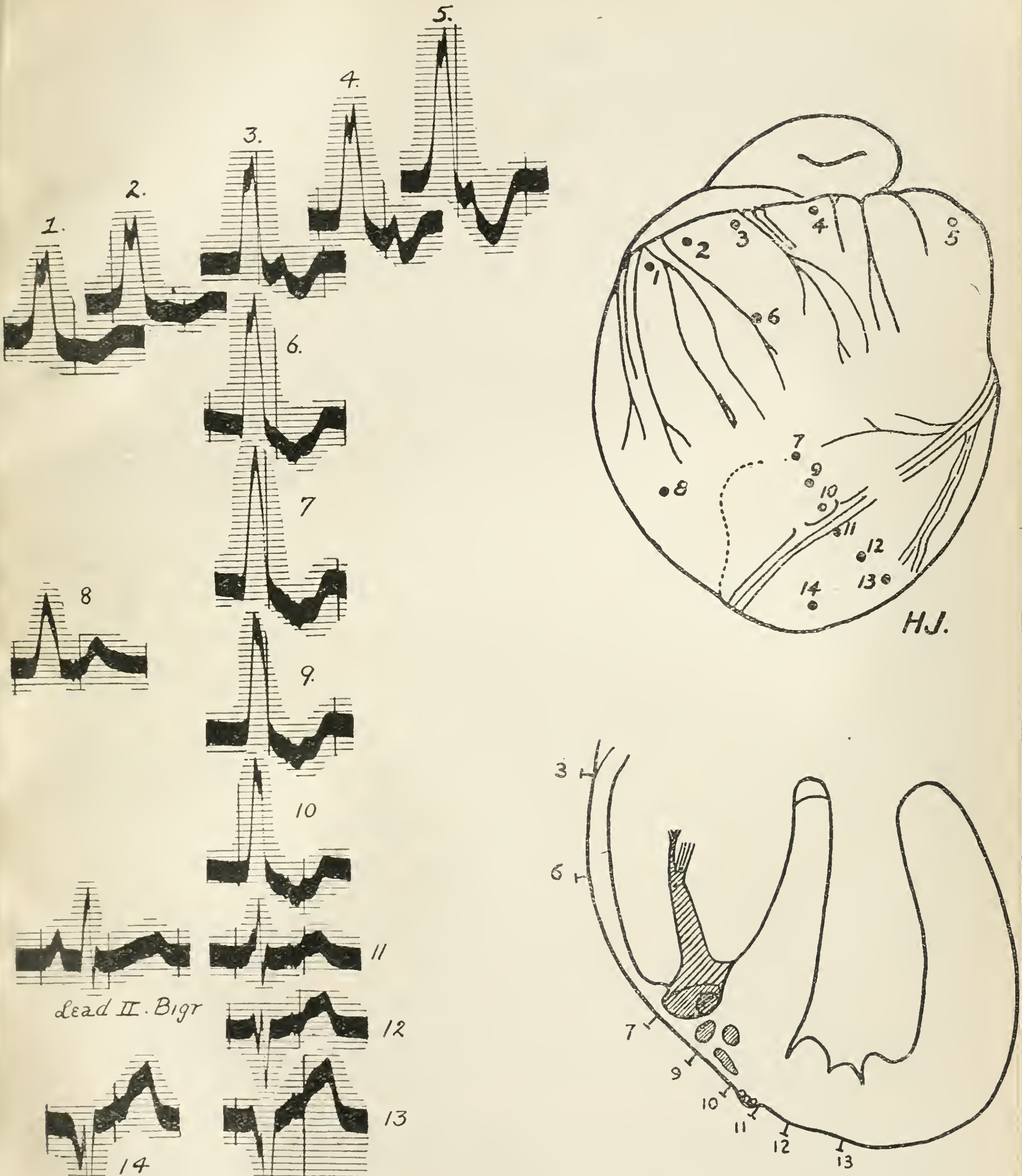


Fig. 10.—(Dog H.J.) A series of curves taken from Lead *II*, and resulting from stimuli applied at the corresponding numbered points in the upper outline drawing of the dog's heart. This outline is to scale and natural size. The lower outline is a section of the same heart, through points 3–13, and is also to scale.

electrodes are moved from the $A-V$ groove (at 3) downwards towards the apex of the heart, the change in form and in magnitude is very slight until the trabeculated region is passed. As the descending branch of the left coronary artery is crossed there is an abrupt change in type; point 11 yields a complex of intermediate type; the complexes from points 12 and 13 have their two chief phases inverted as compared to those obtaining over the right heart. Briefly, the responses from the right ventricle produce electrocardiograms presenting the chief features of *dextrograms*, while the responses from the left ventricle produce electrocardiograms showing the chief features of *levograms*. Now these facts are not new in their entirety; the type of response has been extensively studied by many writers; I may refer to my own summary of such observations,* and to a recent paper by ROTHBERGER and WINTERBERG;† but several points of importance have hitherto escaped notice or explanation.

The first point to which I would call attention is the relation between the length of the interval, signal to first chief phase, and the thickness of the underlying muscle (Table X). In Curves 12 and 13, and preceding the chief or downwardly directed movement, there are very evident preliminary phases of a diphasic character. In Curves 4, 7, 9 and 10 the complex opens with a steep upstroke, and there is no preliminary phase, but small preliminary phases are distinct in all the remaining curves.‡

ROTHSCHILD and I have shown that when the surface of the ventricle is stimulated, the excitation wave quickly spreads to the Purkinje network and reaching it is propagated at an accelerated velocity. The preliminary phases of the present curves are due to the initial spread in the muscle: for, being more or less prominent according as the muscle path is long or short, we may conclude that one of the chief factors governing their appearance is the amount of muscle activated before the Purkinje network begins to convey the impulse. The first chief phase, be it upward or downward, appears immediately after the Purkinje system is involved, and its magnitude is due to quick spread to the relatively large and corresponding muscle area. In this fashion I interpret the relation between the muscle thickness and the interval, signal to chief phase.

The manner of spread when the surface of the ventricle is stimulated is diagrammatised in fig. 11.

The excitation wave spreads from the point of stimulation in all directions, and the muscle mass involved is approximately hemispherical. This hemisphere expands until the whole thickness of the wall is involved; up to this moment the string, at the usual tensions, remains quiescent or shows only minute movements. It is clear that a wide area of muscle wall may be thrown into a state of activity before the recording instrument is affected; this fact requires emphasis because it has to be taken into account in interpreting the natural axial electrocardiogram.

---

* 'Mechanism of the Heart Beat,' London, 1911, p. 146.

† 'Archiv. f. d. ges. Physiol.,' vol. 154, p. 571 (1913).

‡ The preliminary phases are included within the tabulated intervals, the ends of these intervals falling where the preliminary phases terminate.

The next phase of the spread is more complex, the excitation wave having entered the Purkinje substance courses rapidly along the lining of the heart and re-enters the muscle from within outwards. From this time onwards the excitation wave is spreading along two routes: along the old path, which is radial to the point of stimulation, and along the new path from the network. If we consider a point in the region *m*, such a point may be activated by the surface wave, or it may be activated by the wave travelling from within outwards. All points on the surface within the region *mm* will be activated directly from the point of stimulation; all points outside this region will be activated from the network. A little consideration will suffice to show that, when the surface of a ventricle is stimulated, only a limited mass of muscle responds to the ingoing wave of excitation; the chief part of the ventricular wall responds to the outgoing wave. The mass responding to the ingoing wave will depend upon the muscle thickness, but will never be large, because the velocity of conduction in Purkinje tissue is relatively very great. Thus it happens that the direction of travel in the ventricular tissue cannot be controlled by altering the point of stimulation. Stimulation at base or apex of the right ventricle produces the same end result, an excitation wave travelling from within outwards over the greater part of the ventricular substance. This accounts for the general similarity of curves obtained when stimulating a large area of the right, or when stimulating a large area of the left ventricular surface.*

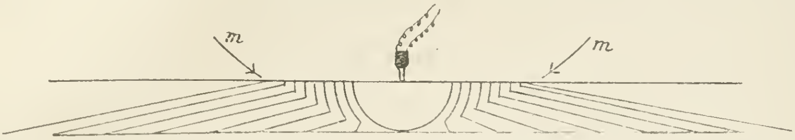


FIG. 11.—A figure illustrating the supposed lines of spread of the excitation wave when a point upon the surface of the ventricle is stimulated.

The second point to emphasise is the relatively abrupt transition from the type shown in Curve 10 to that shown in Curve 12. Clearly such transitions are due to spread into the right Purkinje system on the one hand and into the left Purkinje system on the other.

Finally, when the point stimulated is immediately to the left of the artery (point 11) curves of intermediate type are obtained, and these curves often resemble natural bigrams in the same animal very closely, as ROTHBERGER and WINTERBERG and others have pointed out. The explanation is that the two Purkinje systems are involved almost simultaneously.† To take the present example, the resemblance between Curve 11 and that which stands to the left of it in the figure (*i.e.* the natural bigram) is remarkable. On measurement the point of stimulation was calculated to lie 7 mm. from the nearest Purkinje strands in the right ventricle, and 8 mm. from the nearest strand in the left ventricle. The electrocardiogram, shown in Curve 11, is of dual origin; it is an algebraic summation of a dextrogram and levogram, and has the general outline of the natural bigram. That it fails to reproduce the natural bigram with absolute accuracy is not surprising, for the dextrogram and the levogram which compose it are not quite natural, neither is it probable that they fall with precisely natural time relations to each other. Nevertheless, the experiment corroborates the observations recorded in an earlier section of this communication.

---

* ROTHBERGER and WINTERBERG have suspected a mechanism of the kind described, and briefly discuss its possibility in their article.

† A parallel though less exact explanation was given by the writers cited

Article No. 6

282'

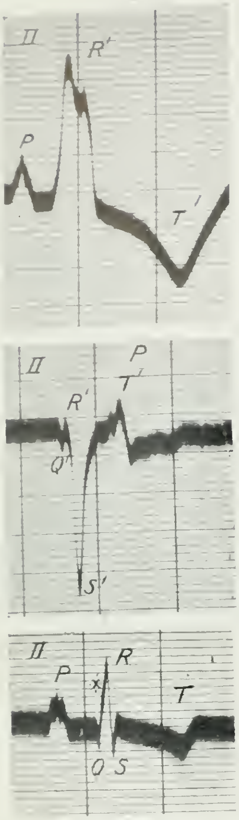

II
R'
P
T'
II
P
R'
T'
Q'
S'
II
P
x
R
T
Q
S

FIG. 12.

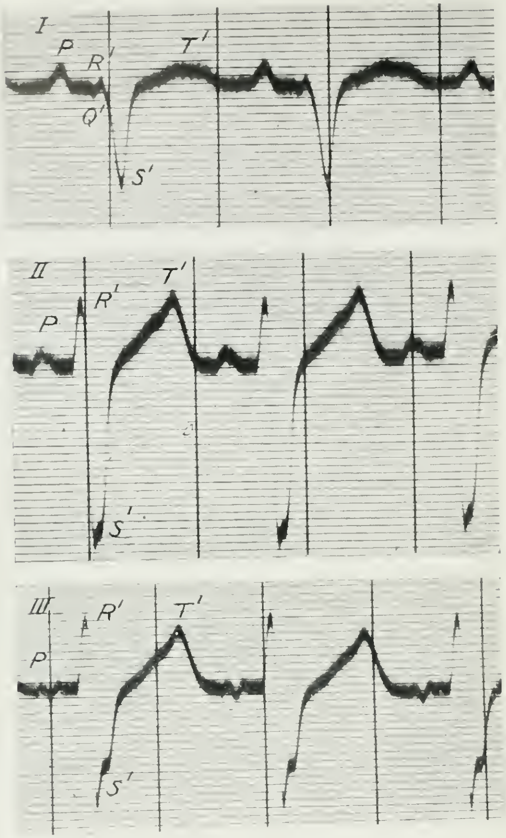
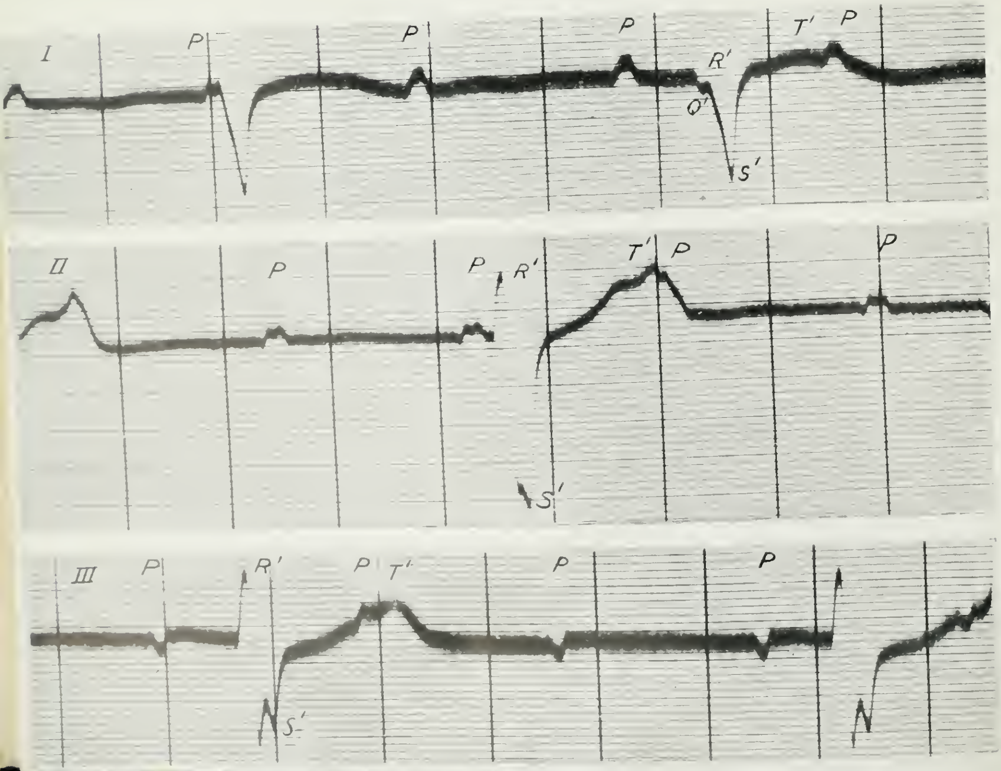

I
P
R'
T'
Q'
S'
II
P
R'
T'
S'
III
R'
T'
P
S'

FIG. 13.


I
P
P
P
R'
T'
P
Q'
S'
II
P
P
R'
T'
P
P
S'
III
P
R'
P
T'
P
P
S'

282^{2}

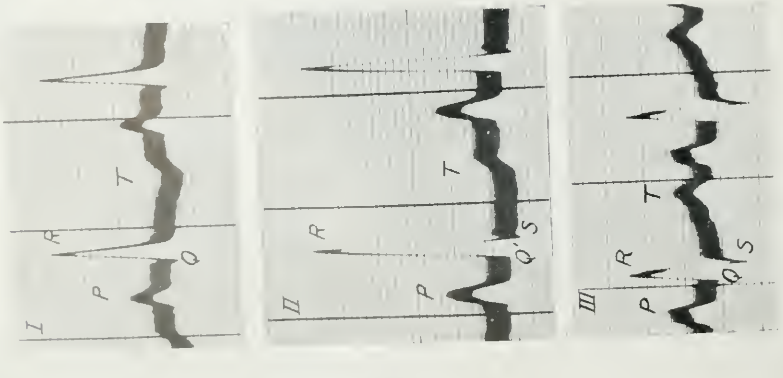


Fig. 15.

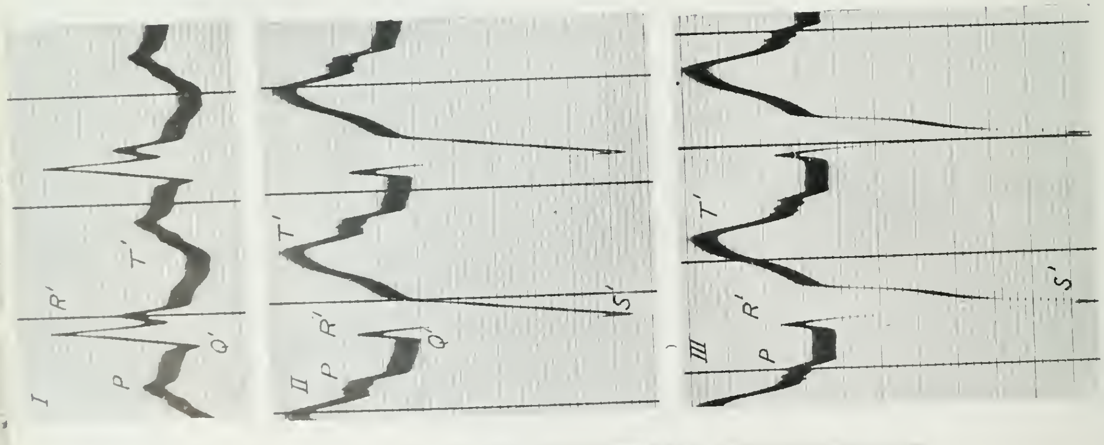


Fig. 16.

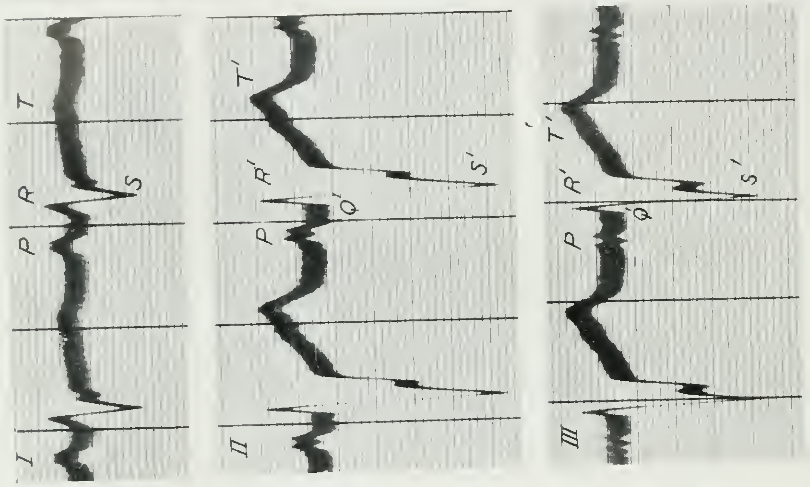


Fig. 17.

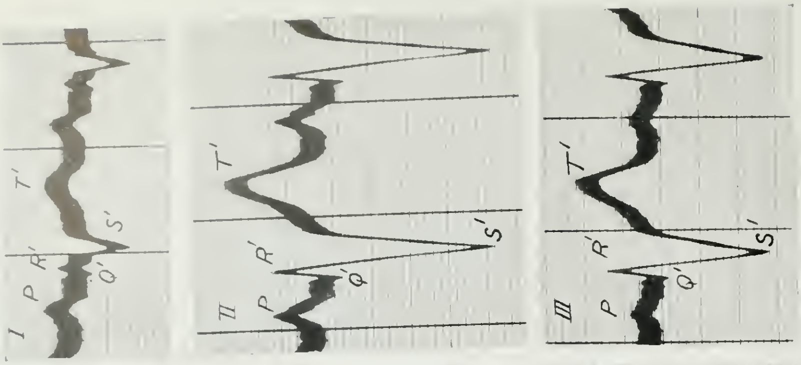


Fig. 18.

282^{3}

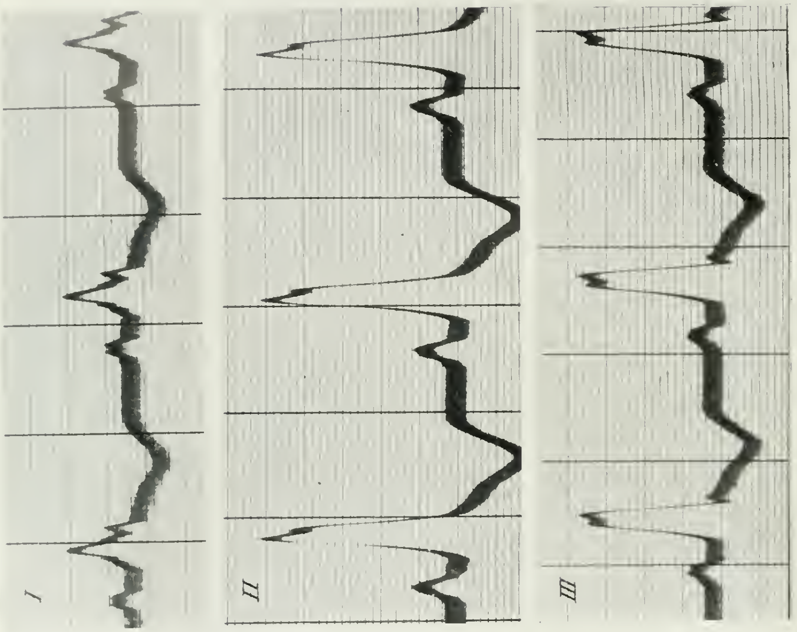


Fig. 20.

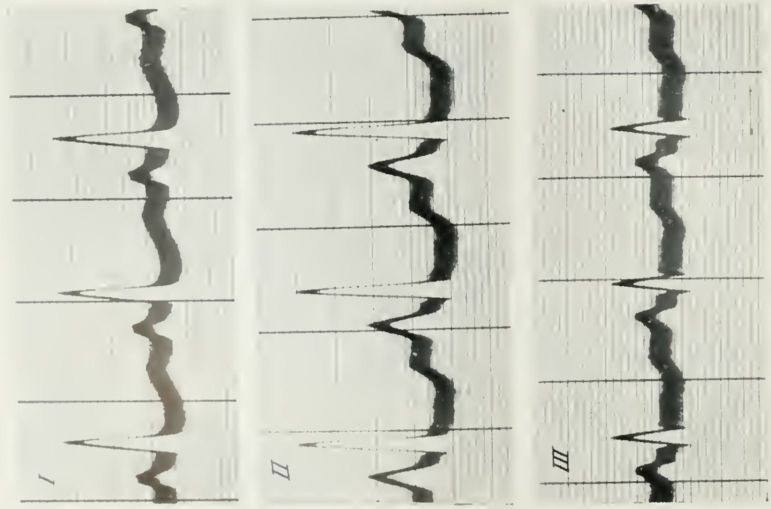


Fig. 19.

EXPLANATION OF PLATES.

Plate 18.

Fig. 12.—(Dog H.H.) Three electrocardiograms from Lead *II*. Top curve dextrogram, middle curve levogram, bottom curve bigram. While the dextrogram and levogram were recorded, complete heart-block was present. These three curves were used in the construction of fig. 2 (p. 251). Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Fig. 13.—Levograms from Leads *I*, *II*, and *III* (Dog I.O.), the result of transection of the right bundle division. Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Fig. 14.—The corresponding levograms, after clamping the *A–V* bundle and producing complete heart-block. The outlines of the ventricular curves in this and the preceding figure are essentially the same. Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Plate 19.

Fig. 15.—(Dog H.W.). Electrocardiograms from Leads *I*, *II* and *III*, chest wall open. Showing the curves of the natural heart beat. Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Fig. 16.—(Dog H.W.). Electrocardiograms from Leads *I*, *II* and *III*, chest wall open. Showing the form of the levogram, obtained by clamping the right bundle division in the same animal. The curves are of the discordant type. Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Fig. 17.—(Dog I.J.) Electrocardiograms from Leads *I*, *II* and *III*, chest wall open; levograms of concordant type produced by transection of right bundle division. Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Fig. 18.—(Dog H.P.) Electrocardiograms from Leads *I*, *II* and *III*, chest wall open; levograms of concordant type produced by clamping right bundle division. Ordinates, approximately, 2 cm. = 3 millivolts; abscissæ = 0·2 sec.

Plate 20.

Fig. 19.—(Dog I.C.) Electrocardiograms from Leads *I*, *II* and *III*, chest wall open. Showing the curves of the natural heart beat. Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Fig. 20.—(Dog I.C.) Electrocardiograms from the same animal and from Leads *I*, *II* and *III*, showing the form of the dextrogram, produced by transection of the left bundle division. Chest wall open. Ordinates, approximately, 1 cm. = 1 millivolt; abscissæ = 0·2 sec.

Part IV.—*The Human Ventricle.*

[PLATE 21.]

Aberrant Contractions.

Shortly after EPPINGER had published his experiments with ROTHBERGER, the same writer recorded some clinical observations in conjunction with STOERK.* In electrocardiograms taken from Leads *I* and *III*, in two patients, they observed abnormalities of the curves which, so they considered, represented defective conduction in the right division of the *A–V* bundle; they obtained *post-mortem* examinations in these two cases, and observed fibrosis of the ventricular septum, which involved the right bundle division in each.

The anomalous curves first depicted by EPPINGER and STOERK are very familiar to those who are accustomed to deal with many cases of heart disease. I have in my collection over thirty examples. Three years ago I described those features of the curves which I regarded as characteristic,† and accepting EPPINGER's explanation I termed the ventricular contractions corresponding to them "aberrant contractions."

CARTER has since published from my laboratory a detailed account of the patients on whom I then based my conclusions,‡ and has included in his paper the curves of 17 of our cases.

In the monograph to which reference has been made, I said that "the chief features of the ventricular complexes are the exaggerated amplitude of excursion, and the prolongation of the initial phases. The deflections which replace the usual *QRS* group have a total duration which exceeds a tenth of a second, and generally comprise more than a third of the whole complex. When the *right* branch of the bundle is at fault, the abnormality declares itself in the broad summit (R') of Lead *I* and in the deep and broad depression (S') in Lead *III*. Damage of the *left* branch portrays itself in the reversed pictures, a deep broad depression (S') in Lead *I*, and a tall and broad summit (R') in Lead *III*." I also pointed out that, while the chief phase is opposite in direction in Leads *I* and *III*, the curve in each lead is in the main diphasic, so that the second phase (T') is invariably in the opposite direction to the chief initial phase. In Lead *II* the curves are not so characteristic; here they are smaller in amplitude than in Lead *III*, but usually follow the general outline of that lead. There is little to add at the present time to this description, with the exception that the chief phase in the damage of the right stem is usually preceded in Leads *II* and *III* by a small or insignificant upward phase, and in Lead *I* by a small downward phase. The curves must be studied in detail and in numbers if the significance of my present observations is fully to be appreciated.

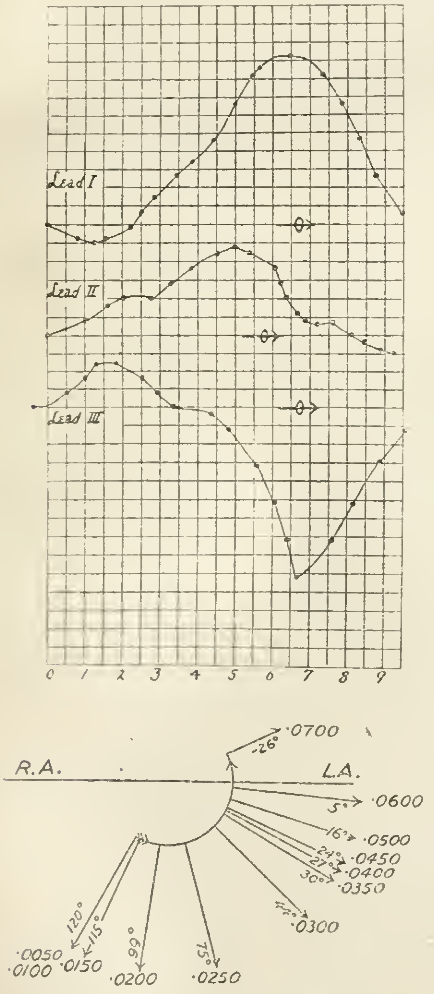
---

* 'Zeitschr. f. klin. Med.,' vol. 71, p. 157 (1910).

† 'Clinical Electrocardiography,' London, 1913, p. 32.

‡ 'Archives of Internal Medicine,' vol. 13, p. 803 (1914).

For my immediate purpose I take two examples, one series of curves which I regard as a characteristic example of a right branch defect in the human subject, the other the solitary example of curves in my possession which I regard as representing a left branch defect (Plate 21, figs. 11 and 14). These two sets of curves, taken during a clinical routine, are unaccompanied by standard simultaneous curves; and to place similar phases of time above each other is not quite so simple as would have been the case had such simultaneous curves been in my possession. The initial



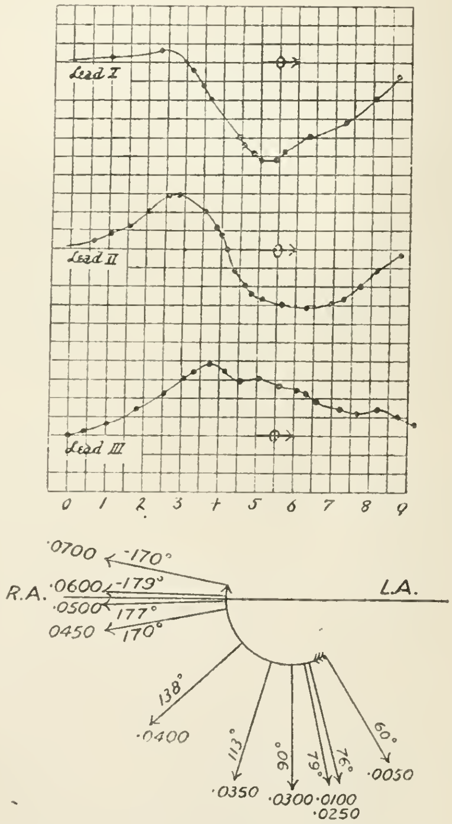


FIG. 1.—A chart of the initial phases of human electrocardiograms, taken from a heart presenting signs of a right bundle branch lesion. Ordinates, 1 cm. = 1 millivolt; abscissæ, 1 cm. = 0·02 sec. A diagram showing the angles of the corresponding electrical axes and the rotation. The actual curves are shown in Plate 21, fig. 11; the values are given in Table I.

FIG. 2.—A similar chart and diagram, taken from a heart presenting signs of a left bundle branch lesion. Ordinates and abscissæ as in fig. 1. The actual curves are shown in Plate 21, fig. 14; the values are given in Table II.

phases of the three leads have been charted in the case of each series, and have been moved relative to each other until a fit, in the sense of an agreement with EINTHOVEN'S formula, has been obtained. This, though an arduous task, is no less certain than the other method, for agreement with the formula can be obtained only when corresponding time phases have been found. The charts are illustrated in figs. 1 and 2, and beneath each chart is a corresponding diagram of the changing direction of the electrical axis. The rotation in the case of the supposed right branch defect (fig. 1) is a very complete and tolerably uniform anticlockwise movement; the rotation in the case of the supposed left branch defect (fig. 2) is a very complete and almost uniform clockwise movement.

TABLE I.—Human Levogram.

	I.	II.	III.	Angle.
sec.				
0	0	0	0	—
0·0050	– 2	2	4	120°
0·0100	– 4	4	8	120
0·0150	– 5	7	12	115
0·0200	– 2	10	~~11~~ 12	99
0·0250	3	~~10~~ 11	8	75
0·0300	9	~~11~~ 12	3	44
0·0350	14	~~15~~ 14	0	30
0·0400	19	~~19~~ 18	– 1	27
0·0450	24 25	22	– 3	24
0·0500	~~33~~ 32	24	– 8	16
0·0600	~~45~~ 44	19	– 25	5
0·0700	~~45~~ 46	3	– 43	– 26
0·0800	30	1	– 29	– 28
0·0900	9	– 5	– ~~13~~ 14	– 50

In this and succeeding Tables the figures representing voltages in the several leads express 0·05 millivolt.

TABLE II.—Human Dextrogram.

	I.	II.	III.	Angle.
sec.				
0·0050	1	2	1	60°
0·0100	1	4	3	76
0·0150	1·5	6·5	5	77
0·0200	2	10	8	79
0·0250	3	14	11	78
0·0300	0	14	15 14	90
0·0350	– 7	10·5	~~18~~ 17·5	113
0·0400	– 14	4	18	138
0·0450	– ~~22~~ 23	– 8	15	170
0·0500	– ~~26~~ 28	– 13	~~15·5~~ 15·0	177
0·0600	– ~~22~~ 25	– ~~15~~ 13	12	– 179
0·0700	– ~~17~~ 20	– ~~15~~ 13	7	– 170

The charted and figured curves with the diagrams of rotated axes may be compared with those of fig. 3 and the angles of Table III, taken from a large Rhesus monkey, in which a lesion of the right bundle branch had been created experimentally.* The comparison leaves little or no doubt that these human curves and those from the monkey have a similar constitution, and convinces us that we may utilise the human curves as a basis in analysing natural human electrocardiograms. At the present time we have an abundance of those curves which represent right

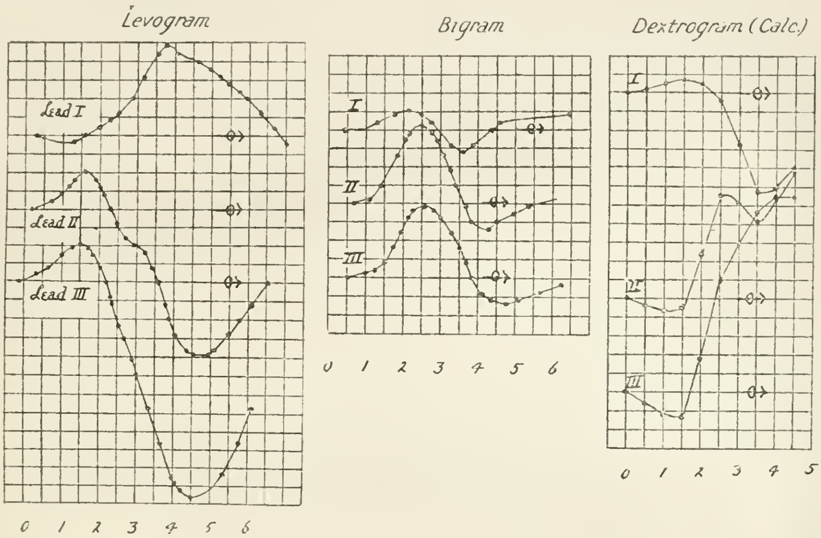


FIG. 3.—A chart showing three sets of curves obtained from a large Rhesus monkey. The series to the left is that obtained after clamping the right division of the bundle. The central series comprises the natural curves obtained from the undamaged heart. The right-hand series shows the calculated dextrograms. Abscissæ and ordinates as in fig. 1. The values of the curves are given in Table III.

TABLE III.—Monkey.

	Levogram.				Bigram.				Dextrogram (calculated).			
	I.	II.	III.	Angle.	I.	II.	III.	Angle.	I.	II.	III.	Angl
sec.												
·0050	– 1	2	3	109°	0	0	0	—	1	– 2	– 3	– 71°
·0100	– 2	5	7·5 7·0	106	0·5	1 1·5	1	71°	2·5	– 3·5	– 6	– 65
·0150	– 0·5	10	10·5	92	3	6·5 7	4	65	3·5	– 3	– 6·5	– 57
·0200	2	6	4	71	4·5	17 17·5	13	76	2·5	11·5	9	78
0225	4	1	– 3	– 16								
0250	6	– 5	– 11	– 57	4	22 22·5	18·5	80	– 2	27·5	29·5	93
0300	13	– 10·5	– 24 23·5	– 56	– 1	15	16	93	– 14	25·5	39·5	110
0350	21	– 17·5	– 38·5	– 57	– 5·5	3	8 8·5	130	– 26·5	20·5	47	124
0400	22·5	– 32·5	– 54 55	– 65	– 3	– 6	– 3	– 120	– 25·5	26·5	52	119
0450	20·5	– 39	– 58 59·5	– 70	1	– 5	– 6	– 81	– 19·5	34	53·5	111

---

* The lesion was confirmed histologically.

branch defects: we have an insufficiency of those which represent left branch defects. Certain conclusions can be recorded nevertheless.

The human levogram—of which we are evidently now entitled to speak with precision—usually begins in Lead *I* in a minute downstroke. *Q* appearing in the human bigram in this lead probably represents spread in the left ventricle. In Leads *II* and *III* the beginning is with a small upstroke; *Q* in the human bigram in Leads *II* and *III* probably represents spread in the right ventricle. In the dog, on the other hand, we have seen it to be a rule that *Q* when it appears in the bigram almost always expresses the commencing activity of the left ventricle.

We may best judge of the time-interval taken for the excitatory wave to complete its circuit in the human heart, from the length of the initial phases of the bigram. The intervals for the separate ventricles are similar, amounting to 0·05–0·07 second. The aberrant curve is a true representation of levogram or dextrogram only so far as the full excursion of its chief phase; that is to say, in the levogram, to the top of *R′* in Lead *I* and to the bottom of *S′* in Lead *III*. The prolongation of the initial phases as a whole is due, as in the dog, to abnormal subsequent spread in the opposite ventricle.

The conclusions in respect of notching of *R* in the dog's electrocardiogram and to other variations in form may be applied equally to the human electrocardiogram.

It will be evident that ROTHBERGER and WINTERBERG'S conclusion that none of these anomalous curves can be regarded as expressing a right or left branch defect* unless the chief phases are in the same direction in Leads *I* and *III* is not sound. It is based upon their findings in the dog. As we have seen, the chief phases of Leads *I* and *III* usually obey this rule in this animal; but even in the dog there is a good deal of latitude in respect of form, and the two chief phases may be opposite in sign. ROTHBERGER and WINTERBERG have fallen into more serious error in extending their conclusion to the human subject; for in man, as in the monkey, the chief phases in Leads *I* and *III* are of opposite sign.

In the human curve, the passage of the excitation wave through the left ventricle is mainly responsible for *R* in Lead *I* and *S* in Lead *III*; while its passage through the right ventricle is chiefly responsible for *S* in Lead *I* and *R* in Lead *III*. The importance of this evident conclusion will be emphasised immediately in our study of the curves of hypertrophy in the human heart.

Curves of Hypertrophy.

Since EINTHOVEN first drew attention to certain abnormal types of electrocardiogram, and with the accumulation of fresh evidences, opinion has gradually hardened to the belief that relative preponderance of the right or left ventricle may be recognised in human electrocardiograms. Recently, the evidences for this view have been summed up and amplified.* Relative preponderance of the left ventricle is represented by an increase in the amplitude of *R* in Lead *I* and of *S* in Lead *III*; while relative preponderance of the right ventricle is represented by an increase in the amplitude of *S* in Lead *I* and *R* in Lead *III*. These are the prominent signs. It is not with this fact but with its explanation that we are concerned at present.

---

* 'Zentralb. f. Herzkrank.,' vol. 5, p. 206 (1913).

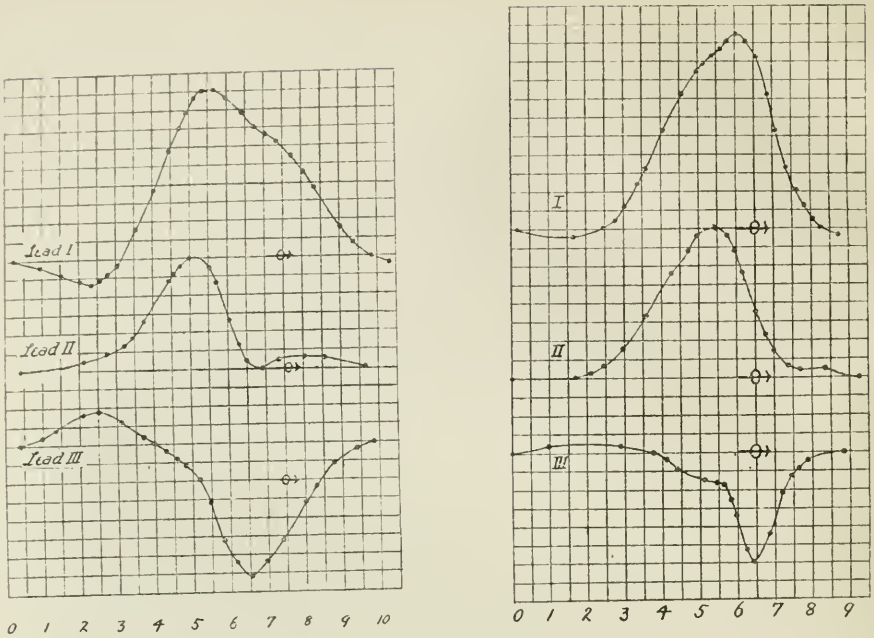
It would seem clear that if a single ventricle becomes enlarged in all its dimensions, the general distribution of the excitation wave throughout it will remain unaltered. But as the mass of tissue involved in the process of excitation is increased, the electric forces liberated will suffer corresponding exaggeration. There appears to be no reason why hypertrophy of the left ventricle should materially modify the general shape of the levogram, but there is reason to anticipate that such a change will magnify the amplitudes of the several deflections. It is to be expected, *a priori*, that the curves from subjects who present preponderance of the left ventricle will exhibit a preponderance of the levogram; the levogram, as we have seen, is mainly responsible for *R* in Lead *I* and for *S* in Lead *III*. An exaggerated *R* in Lead *I*, an exaggerated *S* in Lead *III*, are to be predicted—they are actually discovered—in instances of left ventricular preponderance. Similarly, an exaggerated *S* in Lead *I*, an exaggerated *R* in Lead *III*, are to be predicted—and are actually found—in instances of right ventricular preponderance.† Hypothesis is, to this extent, substantiated by observation. It is further substantiated by more detailed inquiry.

If the curves of left hypertrophy are curves in which there is a notable predominance of the levogram, the rotation of the electrical axis in curves of left hypertrophy should be similar to the rotation of the axis corresponding to the levogram. That is to say, it should be anticlockwise. I have charted the electrocardiograms from a case of chronic renal disease and from two instances of severe aortic disease (figs. 4 and 5), all of which exhibit the signs of left hypertrophy in marked degree. Illustrations of the axis rotations are tabulated in Tables IV and V; in each case there is a conspicuous and steady anticlockwise movement.

---

* Lewis, 'Heart,' vol. 5, p. 367 (1914).

† Considering the conformation of the dextrogram and levogram in the heart of the dog, and the customary differences between these curves and those of the human subject and monkey, the curves of preponderance in one or other ventricle in the dog will not be expressed in quite the same fashion. I venture to predict that, in the dog, preponderance of the left ventricle will show itself, as a rule, by a deepening of *S* in all leads, but especially in Lead *II*, and to a less extent in Lead *III* (less often the changes will be the same as those found in man), and that preponderance of the right ventricle will show itself as an accentuation of *R* in all leads, but especially in Lead *II*, and to a less extent in Lead *III*.



Figs. 4 and 5.—Two charts of the initial phases of the electrocardiograms taken from patients suffering from aortic disease, and exhibiting the signs of left ventricular preponderance. Ordinates and abscissæ as in fig. 1. The actual curves of Chart 4 are shown in Plate 21, fig. 12; these curves are somewhat exceptional, possessing features which probably indicate extreme left preponderance. The method of orientating the curves is illustrated by Plate 21, fig. 10. The standard curve is taken from a constant chest wall lead, the copper electrodes being attached by a stiff and well salted paste. The values of the charted curves are given in Tables IV and V.

Table IV.—Aortic Disease. (Conspicuous Left Preponderance.)

	I.	II.	III.	Angle.
sec.				
0·0050	−0·5	0	0·5	150°
0·0100	−2	0	2	150
0·0150	−4	1	5	139
0·0200	−6	2	8	136
0·0250	−6	3	9	131
0·0300	−2	5	7	106
0·0350	7	~~9~~ 10	3	45
0·0400	17	17	0	30
0·0450	~~30~~ 29	25	−4	22
0·0500	~~40~~ 38	30	−8	19
0·0550	45	28	−17	8
0·0600	43	13	−30	−13
0·0650	38	2	−36	−27
0·0700	~~33~~ 32	1	−31	−29
0·0750	~~29~~ 28	3	−25	−24
0·0800	~~23~~ 20	3	−17	−22
0·0850	~~15~~ 13	3	−10	−17
0·0900	~~7·5~~ 6·5	2	−4·5	−13
0·0950	2	0·5	−1·5	−16

TABLE V.—Aortic Disease. (Left Preponderance.)

sec.	I.	II.	III.	Angle.
0·0100	– 2	0	2	150°
0·0150	– 2	0	2	150
0·0200	– 1	1	2	120
0·0250	1	3	2	71
0·0300	6	~~8~~ 7·5	1·5	41
0·0350	14	15	1	33
0·0400	25	24	– 1	28
0·0450	~~35~~ 35·5	30·5	– 5	23
0·0500	~~43~~ 45	38	– 7	22
0·0550	~~47~~ 48	40	– 8	21
0·0600	~~52~~ 51	33	– 18	9
0·0650	~~47~~ 46	17	– 29	– 9
0·0700	29	12	– 17	– 6
0·0750	~~11~~ 9	3	– 6	– 11
0·0800	3	2	– 1	?11

In the case of right preponderance, the rotation of the axis should resemble the rotation of the dextrogram. It should be clockwise. I have charted the curves in the two instances of mitral stenosis, which exhibit the signs of right preponderance (fig. 6 and Plate 21, fig. 13). The axes are tabulated in Tables VI and VII; in both there is a conspicuous clockwise rotation.

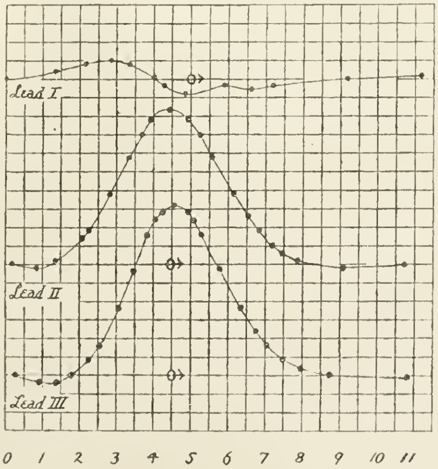


FIG. 6.—A chart of the initial phases of the electrocardiograms from a patient who suffered from mitral stenosis and exhibited the signs of right ventricular preponderance. Ordinates and abscissæ as in fig. 1. The values of these curves are given in Table VI, and the actual curves in Plate 21, fig. 13. The orientation was carried out by the method described in the explanation of figs. 4 and 5 in this and succeeding charts.

Table VI.—Mitral Stenosis. (Considerable Right Preponderance.)

	I.	II.	III.	Angle.
sec.				
0·0050	0·5	–0·5	–1	–60°
0·0100	1	–1	–2	–60
0·0150	~~2·5~~ 3·0	1·5	–1·5	0
0·0200	4	6	2	49
0·0250	5	13	8	68
0·0300	5	~~23~~ 22	17	77
0·0350	2	~~32~~ 31	29	87
0·0400	0	40·5	~~41~~ 40·5	90
0·0450	–3	42	~~46~~ 45	93
0·0500	–4	38	~~43~~ 42	95
0·0550	–3	30	33	95
0·0600	–2	22	24	94
0·0650	–3	13	16	100
0·0700	–2	7	9	102
0·0750	–1·5	2·5	4	112
0·0800	–1	1	2	120
0·0850	–0·5	0	0·5	150

Table VII.—Mitral Stenosis. (Right Preponderance.)

	I.	II.	III.	Angle.
sec.				
0·0050	1	0	–1	–30°
0·0100	3	0	–3	–30
0·0150	6	1	–5	–21
0·0200	~~7·5~~ 7·0	5	–2	14
0·0250	8	10	2	41
0·0300	10·5	~~16~~ 16·5	6	51
0·0350	11	22	11	60
0·0400	10	~~26·5~~ 27	17	69
0·0450	6	~~26~~ 24·5	18·5	76
0·0500	–2	12	~~13~~ 14	98
0·0550	–10	–2	8	161
0·0600	–10	–7	3	?–167
0·0650	–~~7~~ 8	–7	1	?–157
0·0700	–~~4~~ 4·5	–4	0·5	?–156
0·0750	–2	–2	0	?–150

It is also noteworthy that while in the human levogram Q appears and is conspicuous in Lead *I* only, so in curves of left preponderance Q is maximal in the same lead and is, as a rule, confined to it; Q favours Leads *II* and *III* in curves of right preponderance; considering that it is absent from these leads where levograms are concerned but present in them where many bigrams are concerned, it is probable that Q appearing in the bigram in Leads *II* and *III* belongs to the dextrogram.*

---

* Having but a solitary example of dextrograms, the presence or absence of Q in Leads *II* and *III* of these curves cannot be decided conclusively. There appears to be a Q in Lead *III* of fig. 14, but the curves are tremulous and I have not charted it.

In brief, so far as the preliminary phases are concerned, there is between the levogram and curves of left preponderance on the one hand, and between the dextrogram and curves of right preponderance on the other hand, a very close resemblance. In a paper written conjointly with GILDER some three years ago* I gave the amplitude of deflections in a large series of curves taken from normal subjects. Our curves were grouped, so that those which tended to show the signs of right and left preponderance respectively, were collected together. In the first group (Cases 1–25) of our tables were those curves inclining to show right preponderance. The average values of Q in these 25 patients were as follows :—

In Lead *I*	0·2 mm.
In Lead *II*	1·0 ,,
In Lead *III*	1·2 ,,

In the third group (Cases 38–52) of our tables were those curves inclining to show left preponderance. The average values of Q in these 15 patients were :—

In Lead *I*	0·95 mm.
In Lead *II*	0·4 ,,
In Lead *III*	minute.

This analysis confirms the view that Q of the human electrocardiogram may arise in one or other ventricle. If conspicuous in Lead *I*, it is of left ventricular origin ; if conspicuous in Leads *II* and *III*, it is probably of right ventricular origin. If conspicuous in all leads, it probably originates partly in each ventricle.

A Further Comparison.—In the preceding paragraphs, the curves of hypertrophy and of aberrant contractions have been compared, and close points of resemblance between them have been noted. In the fully developed types, and so far as the preliminary phases are concerned, the resemblance is almost, if not quite, complete ; witness the examples placed side by side in Plate 21, figs. 11 and 12.

The origin of the two types is similar in that in each there is a lack of normal balance ; in one case the result of the time factor, the precedence of one ventricle ; in the other case to the factor of mass. How then may the two types be distinguished ? They may be distinguished by their terminal phases. We have seen it to be the rule that in a bundle branch lesion, the curve as a whole is broadly diphasic ; in Leads *I* and *III*, the terminal and prominent phase T' being opposite in sign to the chief phase, R' or S' as the case may be. In the curves of hypertrophy, T does not show this prominence and its direction is not governed in the same manner. Its direction is the direction presented by normal curves, *i.e.*, it is usually upright, though it shows the same variations in amplitude and direction which normal electrocardiograms exhibit. That the distinguishing mark should be found in the latter phases is to be anticipated, for when the excitatory process passes by a single bundle division the ultimate spread to the opposite ventricle is along abnormal routes; the order in which the excitatory process subsides is equally abnormal. On the other hand considerable divergence from the normal order of spread in the case of hypertrophied hearts is hardly conceivable.

---

* 'Phil. Trans.,' B, vol. 202, p. 351 (1912).

A hitherto unnoticed feature of the curves of hypertrophy requires brief discussion. Aberrant curves are remarkable for the duration of their initial phases. Thus in eight normal electrocardiograms the average duration of the initial phases was 0·0784 sec. (Table VIII); in seven unselected examples of right branch defects the initial phases showed an average duration of 0·1396 sec. The initial phases in curves of hypertrophy also show an increased duration, though this is only so where left preponderance is present; the average duration in six cases being 0·1005 sec. It is to be expected that in hypertrophy the spread of the excitatory process will suffer delay, for the layer of muscle to be penetrated is thicker. The reason why the increased duration is confined to hypertrophy of the left ventricle is probably two-fold: firstly, the excess of muscle is greater in left as opposed to right ventricular hypertrophy and the actual increase in muscle thickness is greater; and, secondly, the end of the initial phases of the normal curve (*S* in Leads *II* and *III*) is written by spread in the left ventricle.

TABLE VIII.—Duration of *QRS* Group (in Lead *II*).

Normal.		Left preponderance.			Right preponderance.			Right branch defects.	
Heart rate.	*QRS* group.	Lesion.	Heart rate.	*QRS* group.	Lesion.	Heart rate.	*QRS* group.	Heart rate.	Initial deflections.
	sec.			sec.			sec.		sec.
66	0·0644	Aortic	117	0·0703	Mitral stenosis	119	0·1037	85	0·1721
75	0·0736	,,	118	0·1051	,,	115	0·0738	109	0·1477
97	0·0800	,,	117	0·0848	,,	116	0·0730	92	0·1488
83	0·1038	,,	116	0·1591	,,	65	0·0639	112	0·1225
83	0·0692	,,	111	0·0709	,,	66	0·0625	85	0·1308
86	0·0956	Chronic nephritis	120	0·1129	,,	72	0·0958	90	0·1356
60	0·0782							71	0·1199
75	0·0623								
Averages.									
78	0·0784		116	0·1005		92	0·0788	92	0·1396

Normal Human Curves.

Variations occur in the forms of the electrocardiograms taken from healthy subjects; in some the curves in the three leads incline to the picture of right or left preponderance, as has been stated; other curves are transitional. I have analysed the curves in six healthy subjects and reproduce illustrative charts in figs. 7, 8, and 9 and the values in Tables IX, X, and XI. In instances (fig. 7 and Table IX) where *R* is prominent in all leads, but chiefly so in Lead *II*, and where *S* fails to appear in Leads *II* and *III*, a simple and uninterrupted clockwise movement of the axis is demonstrated. The rotation of the axis is controlled by the right ventricle throughout. Nevertheless these curves may fail to show the predominance of *R* in Lead *III*, as compared to *R* in Lead *I*. On the other hand, they do show *Q* to be confined to Leads *II* and *III*, and *S* to be confined to Lead *I*. They bear several traces of the right preponderance in their outlines and amplitudes but do not exhibit the complete series of signs.

A curious and interesting combination of dextrogram and levogram is illustrated by fig. 8 and Table X. The first phase *Q* is present in Lead *I* only, and the early rotation is anticlockwise and controlled by the left ventricle. During the inscription of *R* in its greater part, the rotation alters its direction and moves definitely but very slightly in a clockwise fashion; over this period the left and right rotations are almost balanced, the right prevailing; finally, during the inscription of *S* the original anticlockwise movement is resumed, the left rotation prevailing. These curves are from an instance of very slight left preponderance within the bounds of normality.

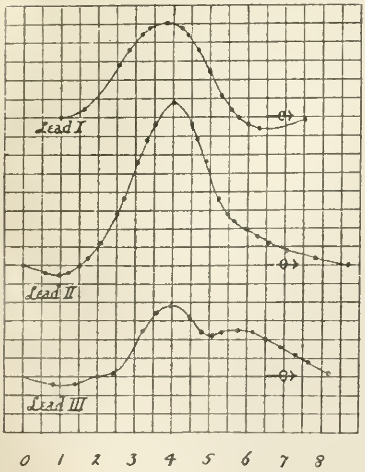


FIG. 7.

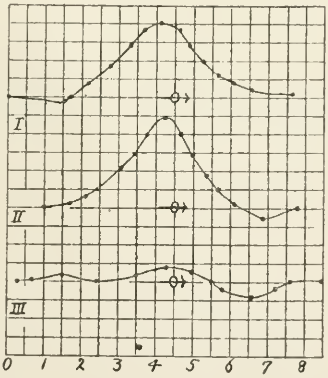


FIG. 8.

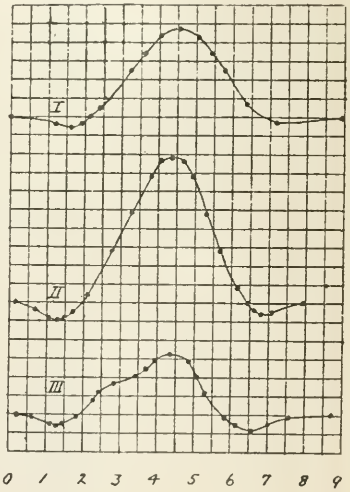


FIG. 9.

FIG. 7.—Chart of the initial phases of a normal human electrocardiogram exhibiting a tendency towards right ventricular preponderance. Ordinates and abscissæ as in fig. 1. The values are given in Table IX.

FIG. 8.—Similar chart from a normal subject showing a very slight left preponderance. At the beginning and end the levogram prevails. In the central part of the chart the dextrogram is predominant.

FIG. 9.—Similar chart from a normal subject showing some preponderance of the left ventricle. At the beginning of the chart the dextrogram prevails, but the remaining rotation is governed by the levogram.

In normal hearts, and while *R* is written and this deflection is prominent in Lead *III*, a clockwise rotation always prevails, indicating predominance of the dextrogram. The regularity of this movement may be disturbed for short periods either at the beginning or at the end of the chart, where the left or anticlockwise rotation asserts itself. Lastly, some normal human curves, which incline towards left preponderance, show an almost uninterrupted anticlockwise rotation of the axis. An example is shown in fig. 9 and Table XI.

Table IX.—Normal.

	I.	II.	III.	Angle.
sec.				
0·0050	0	– 1·5	– 1·5	– 90°
0·0100	0	– 2·5	– 2·5	– 90
0·0150	1·5	0	– 1·5	– 30
0·0200	~~6~~ 5·5	5·5	0	30
0·0250	13	~~14~~ 14·5	1·5	35
0·0300	20	~~27~~ 28	8	46
0·0350	24	~~38~~ 40	16	53
0·0400	25	44	19	55
0·0450	21	~~37·5~~ 37	16	55
0·0500	12	23	11	59
0·0550	2·5	~~14~~ 14·5	12	81
0·0600	– 2	10	12	99
0·0650	– 3	6·5	~~10~~ 9·5	108
0·0700	– 2·5	4·5	~~7·5~~ 7·0	111

Table X.—Left Preponderance within the bounds of normality.

	I.	II.	III.	Angle.
sec.				
0·0100	– 1	0	1	? 150
0·0150	– 1	1	2	120
0·0200	2·5	~~2·5~~ 3	0·5	39
0·0250	~~6·5~~ 6	6	0	30
0·0300	~~11~~ 10	10·5	0·5	32
0·0350	16	~~16~~ 18	2	36
0·0400	20	~~23~~ 23·5	3·5	38
0·0450	19	23	4	39
0·0500	12·5	~~14~~ 15	2·5	39
0·0550	7	7	0	30
0·0600	5	2	– 3	– 70
0·0650	2·5	– 1·5	– 4	– 52

Contents 16-6.
296

Lewis.

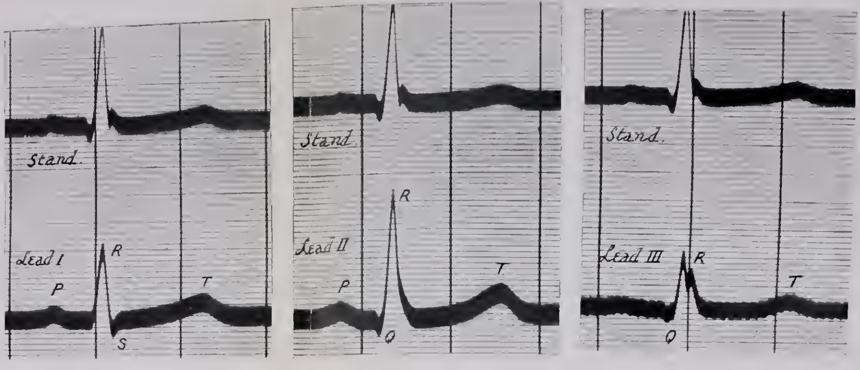


Fig. 10.

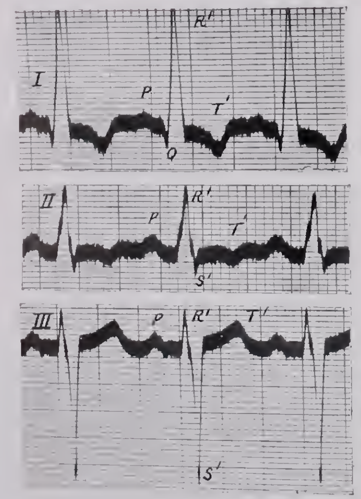


Fig. 11.

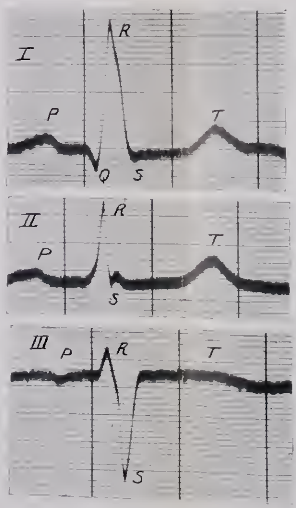


Fig. 12.

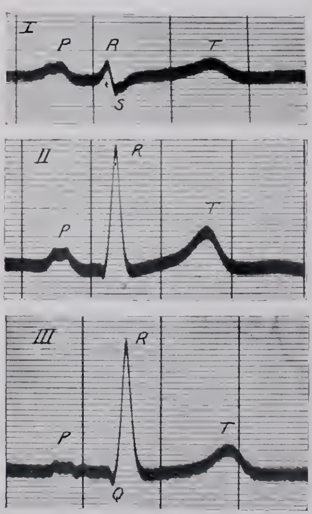


Fig. 13.

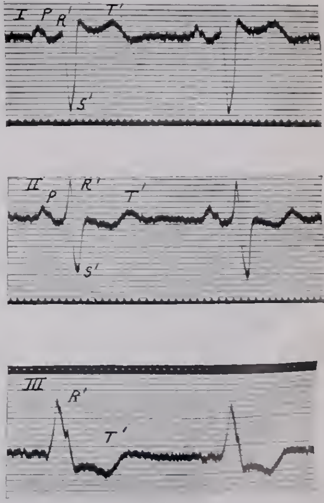


Fig. 14.

Phil. Trans., B, vol. 207, Pl. 21.

Table XI.—Left Preponderance just within the bounds of normality.

	I.	II.	III.	Angle.
sec.				
0·0050	−0·5	−1	−0·5	−120°
0·0100	−1	−~~3·5~~ 3	−2	−109
0·0150	−2·5	−4 4·5	−2	−124
0·0200	−1	0	1	150
0·0250	3	~~9~~ 9·5	6·5	72
0·0300	9	~~18·5~~ 18	9	60
0·0350	16	~~28~~ 27	11	54
0·0400	21·5	~~37~~ 36·5	15	55
0·0450	23·5	~~39~~ 39·5	16	53
0·0500	21·5	33	11·5	50
0·0550	16	19	3	38
0·0600	9	7	−2	18
0·0650	2	−~~1·5~~ 2	−4	−60
0·0700	−0·5	−3	−2·5	−101

EXPLANATION OF PLATE 21.

Fig. 10.—Three photographs from a normal human subject. The upper curve in each is the standard lead, a fixed lead from the chest wall. The lower curves are from Leads *I*, *II*, and *III*. Ordinates, 1 cm. = 1 millivolt; abscissæ, 0·2 sec. These curves were used in constructing the chart of fig. 7.

Fig. 11.—Electrocardiograms from the three leads, indicating a right bundle branch lesion. Ordinates, 1 cm. = 1 millivolt; abscissæ, 0·2 and 0·4 sec. These curves were used in constructing the chart of fig. 1.

Fig. 12.—Three electrocardiograms from a patient suffering from aortic disease, and published for comparison with those of fig. 11. The resemblance is close, so far as the initial phases of the two sets of curves are concerned. Ordinates, 1 cm. = 1 millivolt; abscissæ, 0·2 sec. Used in constructing the chart of fig. 4.

Fig. 13.—Three electrocardiograms from a case of mitral stenosis. Ordinates and abscissæ as in fig. 12. Used in constructing the chart of fig. 6.

Fig. 14.—Three electrocardiograms from a patient in whom a defect in the left bundle branch is supposed to have existed. Ordinates, 1 cm. = 1 millivolt. Time marker in 0·03 sec. Used in constructing the chart of fig. 2.

Part V.—*The Bird's Heart.*

[Plate 22.]

The spread of activity in the bird's ventricle has received no attention hitherto. We know, from the sequence of chamber contraction, that the ventricle receives its impulse from the auricle; the path of the spread is still imperfectly understood. The minute anatomy of the auriculo-ventricular junction was examined by Tawara* who expressed his belief in the existence of a muscular connection between the two chambers. McKenzie and Robertson† stated in 1910 that they could find no specialised musculature at the *A–V* junction of birds; but described a thin leash of cardiac muscle connecting the right auricle with the right ventricle at the right and posterior part of the groove, in the region where the left superior cava has its outlet. Kulb's description‡ differs somewhat from that of these writers; he finds a tract of tissue, in its appearance resembling the bundle of His in mammals, running across the junction at the posterior aspect of the heart, from a point where the right and left auricular walls fuse, and being prolonged forward and downwards to join the musculature of the ventricular septum.

That a muscle tract exists in this neighbourhood seems clear, but its destination is obscure. Its termination in a ventricular arborisation comparable to that of the mammalian heart has not been described. Nevertheless, fibres of very similar constitution to those known as the fibres of Purkinje in the mammalian ventricle have long been known to exist in the bird's heart (Obermeier, Hofmann§). The general distribution of these fibres is also known: they are found subendocardially in the ventricle; but there is no subendocardial concentration, such as is found in the mammalian heart; large collections of the same cells occupy deep positions in the heart wall, especially in the neighbourhood of arterial branches (Hofmann, Tawara, McKenzie and Robertson); these cell collections are indeed conspicuous in sections of the bird's ventricle; the detailed architecture of the columns of cells, if a connected architecture is present, remains untraced.

Flack's experimental work|| tends to confirm the existence of a functional union between auricle and ventricle on the posterior aspect of the groove, for he states that

---

* 'Das Reizleitungssystem,' etc., Jena, 1906.

† 'Brit. Med. Journ.,' vol. 2, p. 1161 (1910).

‡ 'Proc. Internat. Congress,' London, Section III, p. 92 (1913).

§ Obermeier, 'Archiv f. Anat., Physiol. u. wissens. Med.,' pp. 245 and 358 (1867); Hofmann, 'Zeitschr. f. wissens. Zool.,' vol. 71, p. 486 (1902).

|| 'Archiv. Internat. de Physiol.,' vol. 11, p. 120 (1911).

ligatures transfixing the groove in this region produce disturbances of sequence,* and that ligation of other parts of the junction is without effect. These experimental observations still need to be confirmed and extended; especially is it desirable that the experimental lesions should subsequently be explored microscopically.

Method.

The method adopted in the present researches has been a continuation of that employed upon other vertebrata. Fowls and pigeons and also a swan have been anæsthetised with ether; in some instances the animals have been subsequently killed by pithing, respiration being in all instances maintained artificially. The heart has been exposed by a ventral route, the pectoral muscles being disconnected from the sternum, and the latter split and removed. In exploring the ventral surface of the ventricles, the standard curve has been a lead from the right wing to the left thigh. In exploring the lateral and dorsal surfaces, it has been necessary to displace the heart; in these circumstances care has been taken to maintain good tissue contact between the heart apex and the liver or lung, and the electrodes of the standard lead have been adjusted to parts of the body wall in line with the new heart axis. In this manner, and only in this manner, can the usual type of standard curve be maintained.

Direct leads have been taken, one contact lying on the surface of the heart, the other being attached to the left chest wall. Accurate projected or measured drawings have been made of the heart *in situ* in all cases. A single surface only of the heart has been investigated in a given experiment, the standard curve thus remaining quite constant.

The bird's axial electrocardiogram in extracardial leads differs materially in outline from an axial mammalian electrocardiogram; it consists in the main of a prominent downstroke *S* (Plate 22, fig. 13, etc.), followed by a prominent upright and slow deflection *T*. *S* is almost always preceded by a diminutive upward spike *R*. In using the axial curve as a standard, I adopt the summit of *R* (beginning of the downstroke *S*) as the safest point for measurement. The time distance between this point and the onset of the ventricular deflections in the same lead is also measured.

Surface Distribution.

The *ventral surface* is exemplified in the natural size outlines of a swan's heart (fig. 1), a fowl's heart (fig. 2), and a pigeon's heart (fig. 3). The excitation wave first appears in the region of the ventral attachment of the septum (the broken line in these and subsequent figures indicates the boundary of the right ventricular cavity, or the limit of the septal attachment on the right side) in its lowest or apical two-thirds. The readings of the excitation wave traced from this septal line towards the base of the right ventricle increase in quantity; there is with considerable, though not absolute, uniformity a delay in the appearance of surface activity as the base is approached. At the same time, the quantitative differences are small, the whole ventral surface of the right ventricle becoming active within 1½–2 hundredths of a second. In brief, the distribution, so far as it has been described, is generally very similar to that presented by the heart of a dog.

---

* Conclusions in respect of *A–V* conduction in the bird must be drawn circumspectly, considering how easily heart-block follows manipulation of the bird's heart and its peculiar susceptibility to adverse experimental conditions.

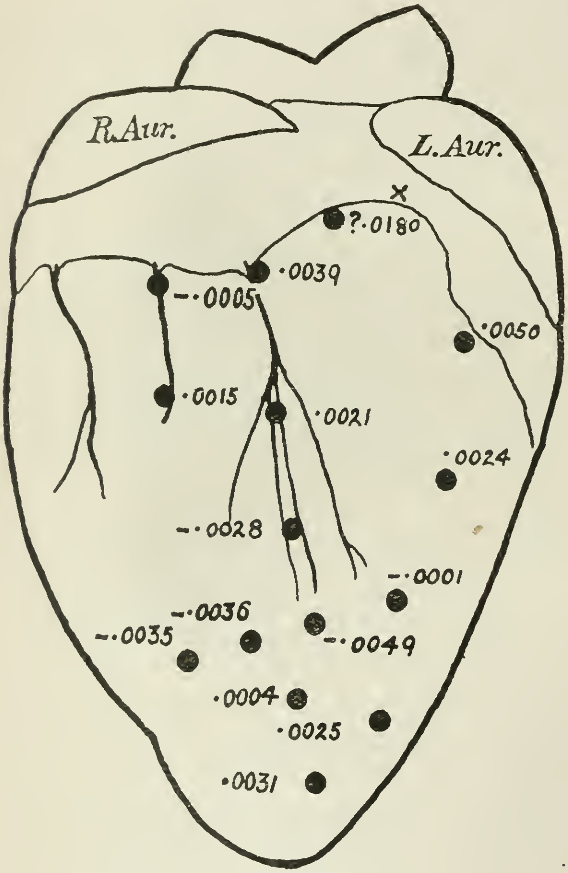


FIG. 1.

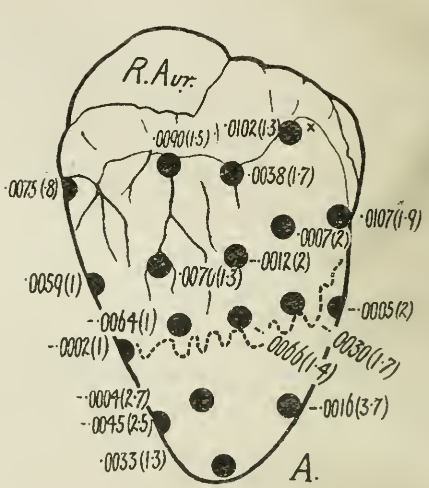


FIG. 2.

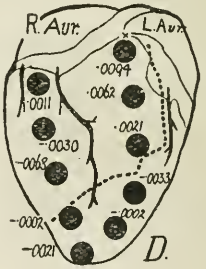


FIG. 3.

FIG. 1.—A natural-size outline of the ventral surface of a swan's heart, showing a number of superficial readings. In this and succeeding figures the size of the contacts is represented as accurately as possible. The conus is marked × in this and subsequent figures.

FIG. 2.—A natural-size projected outline of the ventral surface of a fowl's heart (Fowl *A*), showing a number of surface readings. The broken line in this and succeeding figures represents the limits of the right ventricular cavity.

FIG. 3.—Natural-size projection of the ventral surface of the heart of Pigeon *D*, showing surface readings.

If the readings are traced from the attachment of the septum towards the apex, a similar arrangement is discovered; again there is delay. Thus the septal region stands as the earliest to be activated, the right and left walls on either side of it are activated later. The latest point to become excited has been, with a solitary and unimportant exception, the conus.

The distribution over the *dorsal and right surfaces* of the ventricle is illustrated by the diagrams of two fowls' hearts (figs. 4 and 5) and a pigeon's heart (fig. 6). Though the distribution in the several diagrams lacks uniformity it may be stated, as a general rule, that as we move along the posterior attachment of the septum from *A–V* groove towards the apex of the right ventricle, the readings become earlier; in isolated instances, it is true, they appear to remain almost unaltered. Of this, as of the ventral surface, the broad statement is also true that the values rise as they are traced from septum to left apex. Speaking with all my material in mind, it is the general rule that, as readings are traced along the *A–V* groove from dorsal septal attachment to conus region, the values rise. It is especially to be remarked that the point which overlies the junction of posterior septal attachment and the fat of the *A–V* groove is often relatively late, and is never the earliest point of surface distribution.

The left and left dorsal surfaces are illustrated in the diagrams of two fowls' hearts (figs. 7 and 8) and of a pigeon's heart (fig. 9).

They again show the relative delay at the *A–V* groove, a lesser delay at the heart's apex, while activity first appears in the region of the septal attachments, and especially in the mid-regions of the heart. This aspect of the heart illustrates especially the left ventricle, and the approximation of all its ventricular readings must be emphasised; the whole of its surface is generally covered in about a hundredth of a second.

The Ventricular Surface as a Whole.—In considering the distribution of the surface as a whole, we are at first struck by its general resemblance to that observed in the dog. It resembles the surface distribution in the last-named animal in a particular respect. Many points are supplied simultaneously, and this is especially so over the left ventricle: as is the case over the surface of the dog's heart, the relative values of the readings exclude conduction along muscle bands; where the readings appear in succession the spread has a rapidity which is too great, and the other regions lack the element of uniform succession. It is impossible to review the surface distribution here and in the dog without concluding that the spread is from within* and that the excitatory process is conveyed to the surface by a large number of distinct channels. The chief channel is evidently the septum, for much more often than not the value at a given surface point is great according as it is removed from a septal attachment.

---

* A conclusion which is supported by comparing the intrinsic readings with those of the commencing axial curve and the extrinsic deflections. The axial curve begins in a short upstroke, R, which precedes S by approximately 0·0100 sec. The earliest extrinsic deflections precede S by values varying from 0·0100 sec. to 0·0150 sec. These early deflections are to be explained as resulting from the excitation of the deeper musculature.

I conclude from surface distribution alone that the first spread is to the septum, and that the general spread is fanwise, along radiating paths in the septum, and from its attachment over the free walls of the ventricles. But that the spread is

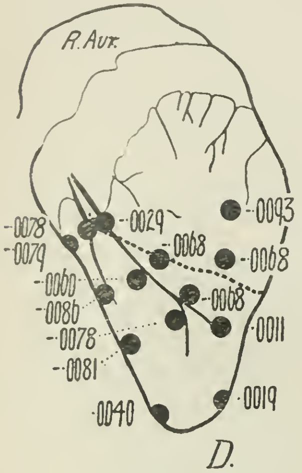


FIG. 4.

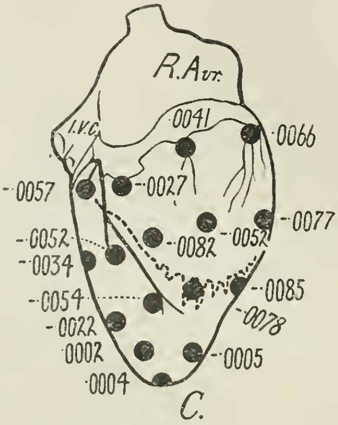


FIG. 5.

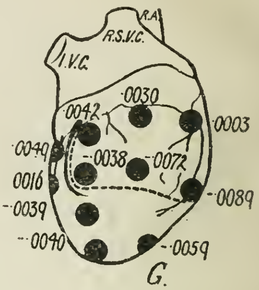


FIG. 6.

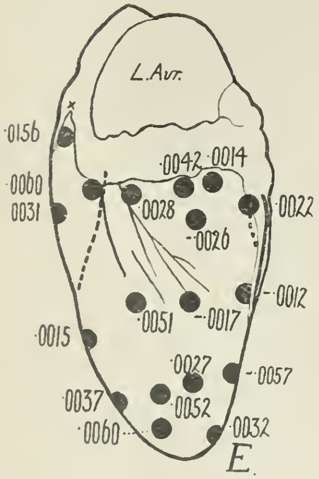


FIG. 7.

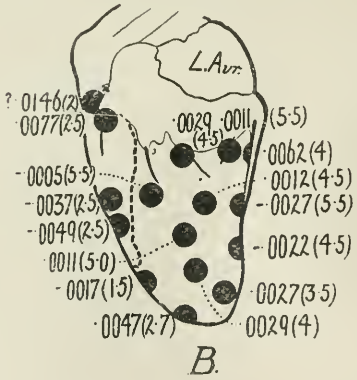


FIG. 8.

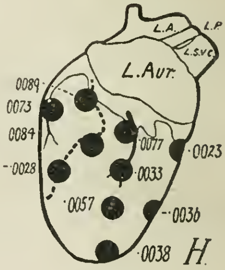


FIG. 9.

FIGS. 4 and 5.—Two natural-size projected outlines of the hearts of Fowls *D* and *C*. Seen from the right side and showing surface readings.

FIG. 6.—Natural-size projected outline of the heart of Pigeon *G*. Seen from the right side and showing readings. *I.V.C.* = inferior vena cava; *R.A.* = right aorta.

FIGS. 7 and 8.—Two natural-size projected drawings of the hearts of Fowls *E* and *B*. Seen from the left side and showing surface readings.

FIG. 9.—Natural-size projected drawings of the heart of Pigeon *H*. Seen from the left side and showing surface readings.

through muscle elements cannot be entertained, so it seems to me, on account of its rapidity; it is through a special distributing system of fibres, as in the dog.

The surface distribution in the bird differs, however, in a noteworthy respect from that discovered in the dog. It differs in that the values over the left ventricle are extraordinarily low. In the dog, as the septum is passed in travelling from right ventricle to left over the ventral surface, the values rise abruptly by some two hundredths of a second or more, and are maintained over the whole left ventricular surface except at the extreme apex. The high values over the left ventricle, as ROTHSCHILD and I were able to show, are the result of muscle thickness and of slow conduction in this muscle, as compared to the conduction rate in the distributing fibres. But in the bird's heart the disproportion in wall thickness between right and left ventricles is much greater than in mammals. How, then, are the low readings to be explained? They are to be explained by an observed peculiarity of the bird's ventricle which accords remarkably with my general hypothesis of the distribution; fibres of Purkinje type penetrate the walls of the ventricle and lie deep in its substance. It is true that occasional strands of the same kind are to be seen in the dog's heart, but the penetration is neither so deep nor so widespread. If, as it is reasonable to conclude, the peculiar fibres of the bird's ventricle, which so closely resemble the Purkinje fibres of the mammalian heart, subserve the same function, then the surface distribution in the bird's heart is not difficult to explain. The reading at a given point on the surface is controlled in the dog by two factors, the length of the Purkinje tract concerned, and the thickness of the underlying muscle. In the bird, I believe that the first factor alone appreciably controls the reading; and, speaking generally, while the value of the reading varies, whether over left or right ventricle, according as the corresponding point lies near or at a distance from the septal attachment, so we may conjecture, if we may not conclude with a reasonable degree of certainty, that the excitation wave spreads by special tracts from above downwards in the septum and through similar special tracts over the free ventricular walls.

Rates of Conduction.

The presence of a special system of distributing fibres is confirmed by the experiences of stimulation and the rate at which an artifically propagated wave of excitation is conducted over various regions of the heart.

Working in the same manner as upon the mammalian heart, I have tested the rate of conduction over right ventricle, left ventricle and septum in a number of birds. In the dog, it will be remembered, the rate of conduction varies, according to the thickness of the muscle layer which overlies the Purkinje network. On the right ventricle rapid, on the left it is slow; for in the former case conduction is chiefly by Purkinje substance and in the latter case by the more slowly conducting muscle. In the bird's heart, where a subendocardial system of conducting fibres is not conspicuous and penetration of the musculature is almost universal, a difference in the conduction rate over the two ventricles is not anticipated. Moreover, it is not found. (See Table I.) The rates of conduction vary considerably, but are high, ranging from 800 to 6000 mm. and more per second. They are no greater over right than over left

TABLE I.

	Right ventricle.			Left ventricle.			Across septum.		
	Interval.	Distance.	Rate of trans-mission.	Interval.	Distance.	Rate of trans-mission.	Interval.	Distance.	Rate of trans-mission.
	sec.	mm.	mm./sec.	secs.	mm.	mm./sec.	secs.	mm.	mm./sec.
Swan A .	0·0063	17·0	2540	0·0020	13·0	6500	0·0232	25·5	1099
Fowl A .	0·0034	13·0	3823	–0·0014	12	∝	0·0186	13·5	726
Fowl D .	0·0104	11·5	1106	0·0089	11	1236			
				0·0187	12	641			
Fowl E .	0·0089	10·0	1123	0·0125	10	800	0·0159	11·0	670
Fowl F .	0·0100	12·75	1275	0·0092	10·0	1087	0·0228	15·0	658
	0·0083	10·00	1205	0·0143	16·0	1119	0·0154	14·0	909
Fowl G .	0·0135	12·00	889	0·0117	13·0	1111			
	0·0107	12·00	1121	0·0111	13·0	1170			

ventricle, and present similar variations over the two chambers. It occasionally happens that with two contacts in line with the point of stimulation, the distal contact first receives the excitation wave, an observation which was made upon the left ventricle of a fowl. This phenomenon has never been encountered in the dog except over the right ventricle; it is attributed in both instances to the presence of a favourable Purkinje channel of conduction to the distal contact. The rates across the septum in the bird are uniformly low, as in the dog's heart, suggesting that, to some extent at least, the supply of right and left ventricle is separate, as it unquestionably is in the mammal.

THE ELECTRICAL AXIS AND THE MEANING OF THE AXIAL ELECTROCARDIOGRAM IN EXTRACARDIAL LEADS.

Records have been taken from two fowls and two pigeons, using pairs of contacts arranged as a triangle. Non-polarisable contacts were placed beneath the skin at the base of each wing; a third contact was placed immediately below and deep to the free end of the breast-bone in the middle line. The curve yielded by each lead (*I*, *II*, *III*) from a pair of contacts was taken simultaneously with a standard curve from a contact at the base of the neck and right chest wall. Subsequently, and after washing out the blood, formalin was injected into the vessels and the heart hardened *in situ* without breaking the chest wall, the bird being then cut in the plane of the electrodes. The diagrams which accompany the charted curves accurately represent the relations of the hearts to the contacts; the arrows and numerals indicate the direction and numbers of the leads. The angles expressing the electrical axis are related to the base line of Lead *I*.

The electrical axis of the bird's heart generally moves, during the initial phases of the activity, through approximately 180°. For a first and short phase of about 1/100 second the axis is in the line of the body and is directed downwards, indicating a general movement of the excitation wave in this direction. These events precede the chief downward movement (*S*) in these leads and correspond to diminutive upward phases (*R*) which are the rule in axial curves from the bird's heart; they occur therefore immediately prior to the appearance of the excitation wave on the surface of the heart; or correspond, in some instances, to its earliest appearance on the surface in the region of the septal attachments.

In one of the fowls these initial phases were inconspicuous and do not figure in the corresponding Table (Table II, Fowl I). There may be, as in the other fowl (fig. 10 and Table II, Fowl H), a gradual rotation of the angle, but usually (fig. 11 and Table III) there is a more abrupt displacement, and the electrical axis turns almost at once through 180° from 90° to −90°, where it remains. In Leads *II* and *III* deep deflections (S) are the resultants, while the curve in Lead *I* remains almost

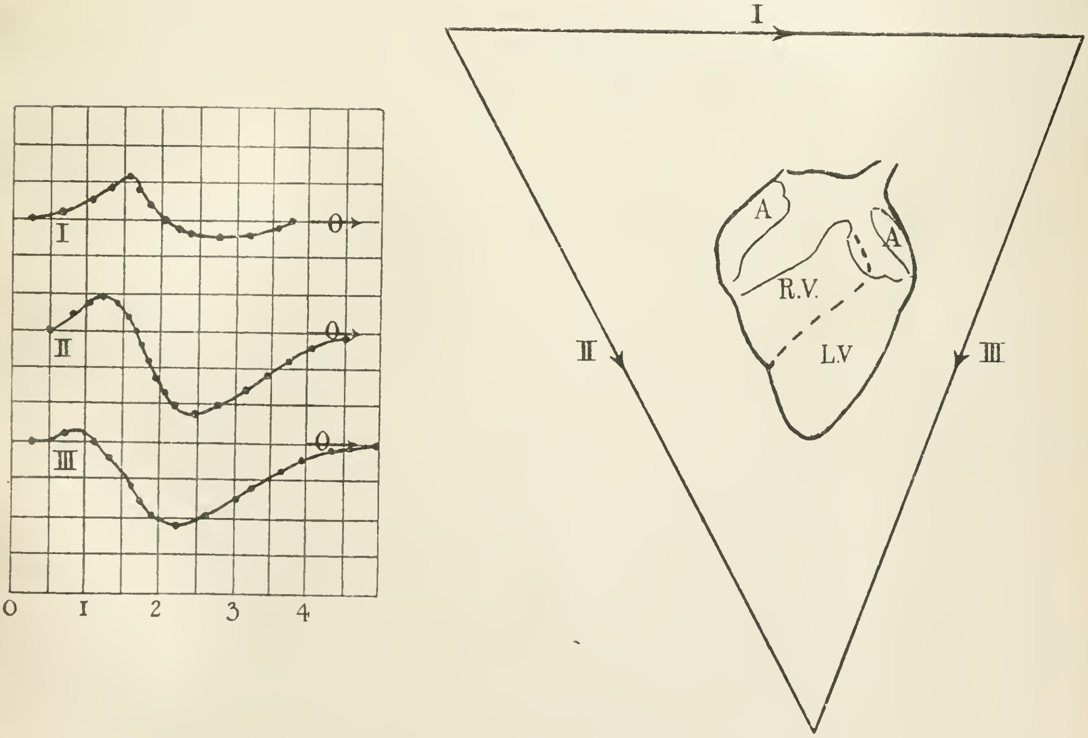


Fig. 10.—(Fowl H.) Chart of the initial phases of the electrocardiogram in Leads *I*, *II*, and *III*. Ordinates, 6 cm. = 1 millivolt; abscissæ, 1 cm. = 0·01 sec. The corresponding electrical axes are given in Table II.

And a diagram of the heart (natural size), showing its exact relation to the leads. The ventral surface of the heart was just within the plane of the contacts.

TABLE II.

	Fowl H.				Fowl I.			
	I.	II.	III.	Angle.	I.	II.	III.	Angle.
sec.								
0·0050	0·5	0	0	—	2·5	–1	–3·5	–46°
0·0080	1	2	1	60°	4	–8	–~~11~~ 12	–71
0·0100	2	3	1	49	1·5	–14	–~~16~~ 15·5	–85
0·0130	4·0	~~4·5~~ 3·0	–1	16	–1	–17	–~~17~~ 16	–93
0·0150	~~5·5~~ 6·0	3	–3	0	–1	–16	–15	–93
0·0180	3·5	–3	–~~8~~ 6·5	–57	–1	–13·5	–12·5	–94
0·0200	1	–7	–~~10~~ 8	–83	–1	–11	–10	–95
0·0230	–1	–11	–~~11~~ 10	–95	–1	–9	–8	–96
0·0250	–2	–~~11~~ 12	–10	–99	0	–~~7~~ 6	–6	–90

NOTE.—In this Table the potential values under columns I, II, and III are expressed in sixtieths of a millivolt. This Table and the succeeding Table have been constructed from the original charts; the corresponding published figures have been redrawn and are not precisely accurate.

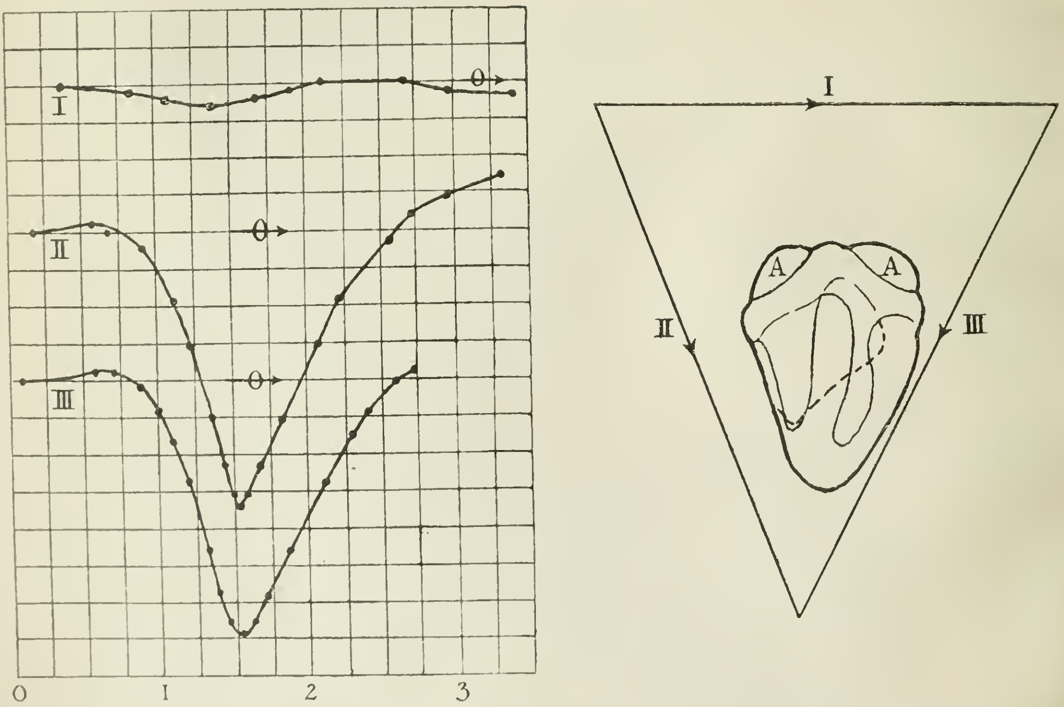


FIG. 11.—(Pigeon J.) A chart showing the initial phases of the electrocardiogram in Leads *I*, *II*, and *III*. Heart rate, 180 per minute. Ordinates, 2 cm. = 1 millivolt; abscissæ, 2 cm. = 0·01 sec. The corresponding electrical axes are given in Table III.

And a diagram (natural size) showing the heart and its exact relation to the leads. A section in the plane of the contacts opened the cavities of both ventricles, the outlines of which are shown. The broken line indicates the extreme limits of the right ventricular cavity.

TABLE III.

	Pigeon J.				Pigeon K.			
	I.	II.	III.	Angle.	I.	II.	III.	Angle.
sec.								
0·0050	0	1	1	90°	0	1·5	1·5	90°
0·0075	−1	0	1	? 150	0	1	1	? 90
0·0100	−1·5	−~~5·5~~ 6·0	−4·5	−104	0	−16	−~~15~~ 16	−90
0·0125	−2·5	−~~19~~ 19·5	−17	−97	−2	−39	−37	−93
0·0150	−2·5	−~~36~~ 36·5	−34	−94	−6	−63	−57	−95
0·0175	−1·5	−29	−27·5	−93	−5	−~~43~~ 42	−37	−96
0·0200	−0·5	−~~19~~ 18·5	−18	−91	−2·5	−~~19~~ 19·5	−17	−97

NOTE.—In this Table the potential values under columns I, II, and III are expressed in 0·05 millivolt (Pigeon J) and 0·075 millivolt (Pigeon K).

isoelectric. The deflections (S in Lead *II* and *III*) speak for a general movement of the excitation wave in the upward direction in the axis of the body.

Where the movement is in short steps, the succession is regular and anti-clockwise, and the curves in Leads *I*, *II*, and *III* present a considerable resemblance to those seen in the dog, where the right bundle division is not conducting. I read the rotation of the electrical axis as expressing an alteration in the average direction in which the fibres are activated, and find that it confirms my view that the excitatory process first spreads downwards through the septum, and later upwards through the free walls. The abrupt passage from an angle of 90° to an angle of −90°, as opposed to a more gradual passage through a wider angle in the mammal, is to be explained by the arrangement of the special distributing tracts. In the mammal these are sub-endocardial, and the spread in the septal and free wall of the ventricle is away from the endocardial surface; in the bird, where the sub-endocardial concentration is not conspicuous, and where the special cells are found abundantly in the substances of the musculature, the path of the spread may be expected to be less at right angles to the wall, and more in line with it.

Thus the rotation of the electrical axis as calculated from extracardial leads from the bird's heart is very similar to that calculated from the levogram of the dog's heart. The bird's axial curve is in the main an expression of left ventricular activity. In the dog (Table IV) the average ratio of the weights of the separated right and left ventricles is as 1 is to 1·8; in the bird's heart the same ratio is as 1 is to 3·3 (Table V). The bird's axial curve differs from the levogram of the dog in several important respects however.

1. There is no preliminary downward deflection *Q*; the spread in the upper part of the septum is not chiefly from left to right or right to left as in the dog and man; it is chiefly from above downward.

2. The initial phases, *R* and *S*, have relatively a short duration (Table VI), amounting usually to 0·0300 or 0·0400 second, and constituting as a rule no more than one-fourth or one-eighth of the whole ventricular complex (in the dog they constitute approximately one-third of the full complex). The rapidity of spread through special intramural tracts is partly responsible for this shortening of the initial phases, which is also conditioned by simultaneous rather than subsequent spread through the right ventricle.

3. The axis tends to depart but little from the vertical; and this almost vertical set of the axis, as has been stated, is attributed to spread in the line of the septum or ventricular wall.

Table IV.—Dog's Ventricular Weights.

No.	Right.	Left.	Septum.	L/R.
	grm.	grm.	grm.	
1	22·0	46·5	11·7	2·11
2	30·5	50·0	13·0	1·64
3	25·7	43·5	10·5	1·69
4	25·0	45·5	15·5	1·82
5	12·0	25·5	7·5	2·12
6	47·5	75·0	22·5	1·58
7	22·5	37·5	14·0	1·67
8	22·5	40·5	19·0	1·80
9	53·5	92·0	28·5	1·74
			Average	1·80

Table V.—Turkey's Ventricular Weights.

No.	Right.	Left.	Septum.	L/R.
	grm.	grm.	grm.	
1	5·2	16·5	5·8	3·17
2	4·1	12·5	4·5	3·05
3	5·1	15·6	4·6	3·06
4	4·6	12·6	4·2	2·74
5	4·6	17·5	5·4	3·80
6	2·5	9·4	4·0	3·76
			Average	3·26

It is improbable that the right ventricle materially contributes to the curves; where it does contribute it contributes in the same direction as does the left ventricle. While the excitation wave is rising through the free wall of the left ventricle it is also rising through the free wall of the right ventricle. The absence of a conspicuous

R deflection in the bird is to be explained by the fact that the whole free wall of the right ventricle is activated in an upward direction.

TABLE VI.

	Average heart rate.	R.	Summit of R.	Bottom of S.	Return of S.	Latest intrinsic reading.	End of T (rate varying).	Length of intrinsic.
Pigeon C . .	221	−0·0069	0·0000	0·0094	0·0218	0·0101	0·1618	
,, D . .	280	−0·0095	0·0000	0·0121	0·0243	0·0094	0·1213	
,, E . .	233	—	—	—	—	0·0042	—	
,, F . .	179	−0·0079	0·0000	0·0123	0·0270	0·0058	0·1670	
,, G . .	213	−0·0076	0·0000	0·0130	0·0304	0·0049	0·1510	
,, H . .	167	−0·0078	0·0000	0·0122	0·0310	0·0089	0·1681	
,, I . .	105	?	0·0000	0·0160	0·0382	0·0067	—	
Swan . . .	257	−0·0060	0·0000	0·0117	0·0310	?0·0189 ?0·0050	0·1509	
Fowl A . .	122	−0·0126	0·0000	0·0132	0·0357	0·0107	0·2572	
,, B . .	132	−0·0109	0·0000	0·0133	0·0378	?0·0146 ?0·0077	0·3048	
,, C . .	161	−0·0131	0·0000	0·0105	0·0310	0·0066	0·2493	
,, D . .	210	−0·0108	0·0000	0·0131	0·0279	0·0040	0·1921	
,, E . .	155	−0·0055	0·0000	0·0118	0·0286	0·0156	0·1615	

The general hypothesis of spread in the bird's heart may be summed up diagrammatically (fig. 12). In constructing this figure I have chosen readings which may be taken as fairly representing the regions of the heart seen in this coronal section, and have added to each 0·0100 second* to render them compatible with the plotted curves of the charts (figs. 10 and 11).

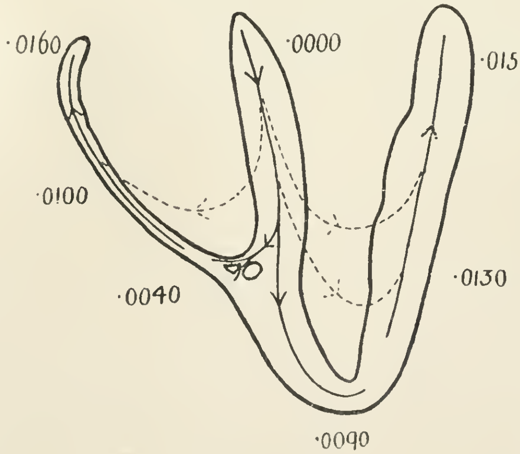


FIG. 12.

Now, the initial phases of the bird's axial curve have a duration which may be taken approximately at 0·0350 second (see Table VI). Of this time interval the first

---

* The distance R—S.

0·0200 second is covered by the spread of the excitation process in the ventricle. Add to this 0·0100 or 0·0150 second as the time taken for the full development of surface potential, and the sum total, 0·0300 or 0·0350 second, is in sufficiently close agreement with the length of the initial phases. The general rule, that the time interval covered by direct readings from the heart is the same as the length of the initial phases, applies to the bird's heart, as to the heart of other vertebrata.

EXPLANATION OF PLATE 22.

Fig. 13.—(Fowl C.) Simultaneous electrocardiogram from Lead *II* and curve from direct ventricular lead. Illustrating the method of obtaining direct readings from the heart. The curve from the direct lead shows a minute extrinsic and a sharp and tall intrinsic deflection. Ordinates, for Lead *II*, approximately 1 cm. = 1 millivolt ; for the direct lead 1 mm. = 1 millivolt ; abscissæ = 0·2 sec.

Fig. 14.—Similar curves from a swan. In the direct lead, the extrinsic deflection is a distinct and sharp downward deflection and this is followed by the usual and prominent intrinsic spike. Ordinates and abscissæ as in fig. 13.

Fig. 15.—(Pigeon J.) Leads *I*, *II* and *III*, each taken simultaneously with a standard curve. The latter was from the top of the sternum to the lower part of the right chest wall. Ordinates of the three leads, approximately, 1 cm. = 1 millivolt ; abscissæ = 0·2 sec.

Fig. 16.—(Fowl I.) Similar photographs from Fowl I. Ordinates of the three leads, approximately, 3 cm. = 1 millivolt ; abscissæ = 0·2 sec.

Article

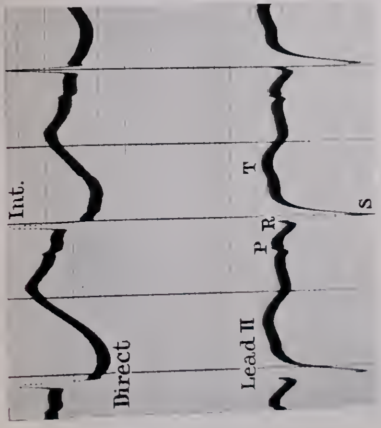


Fig. 13.

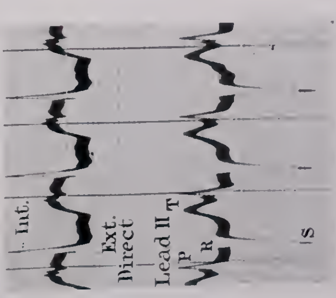


Fig. 14.

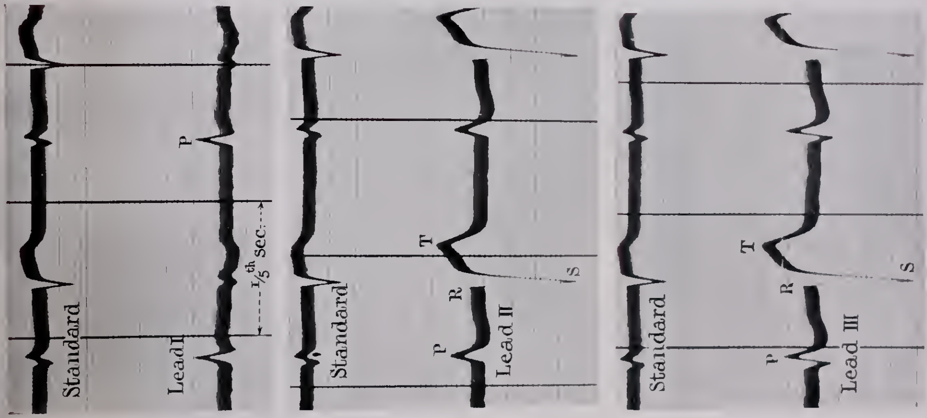


Fig. 15.

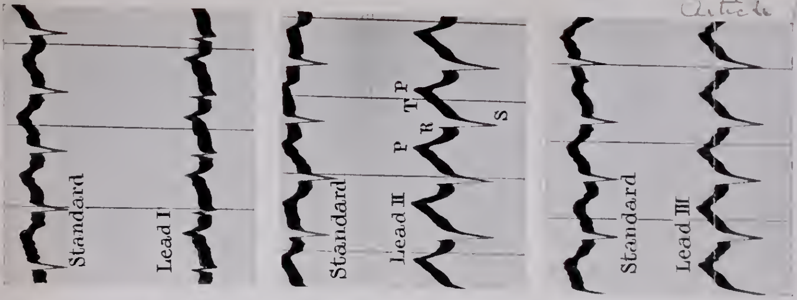


Fig. 16.

0·0200 second is cov
Add to this 0·0100 o
surface potential, an
agreement with the
interval covered by
initial phases, applies

Fig. 13.—(Fowl C.)
direct v
readings
extrinsic
approxim
abscissæ
Fig. 14.—Similar cu
is a disti
usual and
Fig. 15.—(Pigeon J.
standard
part of t
1 cm. =
Fig. 16.—(Fowl I.)
leads, ap

Section of Pathology.

President—Dr. W. BULLOCH, F.R.S.

The Nature of Serum Antitrypsin and its Relation to Autolysis and the Formation of Toxins in Infection and in Anaphylaxis.[1]

By F. H. TEALE and E. BACH.

From the Research Laboratories, University College Hospital Medical School, London.[2]

(I) NATURE AND PROPERTIES OF ANTITRYPSIN.

(*A*) *The Antitrypsin of Egg White.*—(*a*) Relation to lipoids; (*b*) isolation and properties of antitrypsin.

(*B*) *Serum Antitrypsin.*—(*a*) Action of lipoid solvents on (1) dried serum, (2) ordinary serum; (*b*) endeavours to isolate serum antitrypsin; (*c*) soaps as antitrypsin agents; (*d*) inhibition of antitrypsin by (1) acids and alkalis, (2) temperature, (3) incubation; (*e*) adsorption of antitrypsin; (*f*) mode of action of antitrypsin; (*g*) antitrypsin in albumen fraction of serum.

(II) AUTOLYSIS OF THE SERUM.

(*a*) Result of incubation without destruction of the antitrypsin; (*b*) result of incubation after destruction of the antitrypsin; (*c*) sensitiveness of the ferment to (1) de-antitrypsinizing reagents, (2) reaction, (3) temperature; (*d*) adsorption of the ferment.

---

[1] At a meeting of the Section, held December 2, 1919.

[2] The expenses of this research were defrayed by a grant by the Medical Research Committee.

(III) AUTOLYSIS OF SOLID TISSUES.

(*A*) *Liver.*—(*a*) Importance of complete bacteriological sterility; (*b*) effect of reaction; (*c*) effect of bacteria; (*d*) effect of phosphorus; (*e*) effect of bacterial toxæmia.

(*B*) *Leucocytes and Marrow.*

(IV) RELATION OF ANTITRYPSIN TO TISSUE AUTOLYSIS.

(*a*) Is there a tissue antitrypsin?

(*b*) Influence of serum antitrypsin in tissue autolysis: (*a*) liver, (*b*) marrow, &c.

The relation of the above experiments to (*a*) bacterial toxæmia, (*b*) anaphylaxis, (*c*) anaphylotoxin, (*d*) parasitism and pathogenesis of bacteria will be discussed later in a following paper dealing with the variations of antitrypsin in disease and anaphylaxis.

SERUM ANTITRYPSIN AND AUTOLYSIS.

The following experiments on antitrypsin and autolysis were carried out preliminarily to an investigation into the variation of antitrypsin in bacterial infection and anaphylaxis and its relationship to the symptoms of toxæmia.

Various investigators have been struck by the uniformity of the main manifestations of bacterial toxæmia in animals of the same species and have come to the conclusion that the symptoms are due not to specific bacterial endotoxins but to a non-specific poison formed (1) either from the bacteria themselves by the action on them of antibodies and complement (Teale and Embleton [1], Vaughan [2]); or (2) from the plasma of the infected animal (Jobling and Petersen [3]). These last authors believe that bacteria can adsorb antitrypsin and so allow the plasma proteolytic enzymes to give rise to proteose-like toxic bodies from the animal's own body fluids. Further Wright [4] in his work claims to have shown that certain bacteria can only grow in the tissues, &c., and produce toxins when the local adsorption of the serum antitrypsin is sufficiently great to allow of auto-digestion of the tissue fluids, and the consequent production of sufficient split products to allow these bacteria to grow.

Lastly, our experiments on the changes in the nitrogen content of the blood in bacterial infection showed a great increase of non-coagulable nitrogen, chiefly, in fact almost wholly, in the form of urea, and so it became necessary to investigate the influence of the serum antitrypsin on tissue autolysis; and also to determine whether a special tissue antitrypsin was present or not.

HISTORICAL.

Hildebrandt [5] made the first observation that the unheated sera of all animals and also egg albumen possess a marked resistance to the action of proteolytic enzymes. Hahn [6] made a similar observation a few years later. According to Levine and Sankey [7] this antitryptic property is also possessed by the various tissues and organs of the body. Weinland [8] demonstrated that certain intestinal worms contained a powerful antitrypsin, to which he attributed their ability to live bathed in the intestinal digestive juices. This observation was confirmed by Hamill [9] who isolated the antitrypsin of intestinal worms and studied its properties. He showed that the antitrypsin was not a lipoid or a lipoidal combination. The antitrypsin did not destroy the trypsin but inhibited its action for a time until it was itself destroyed by the trypsin. Cathcart [10] showed that the antitrypsin is connected with the albumen fraction of the serum.

With regard to the nature of antitrypsin there are various views:—

(1) That it is a true antibody in the sense of Ehrlich—(Achalme [11], Jochmann and Kantorowicz [12], Mayer [13], Eisner [14], and Wiens [15]). Mayer states his belief that the antitrypsin is an antibody provoked by the ferments of the cells as antigens. Eisner [14] and Wiens [15] believe that the antigen is the leucoprotease of the polymorphonuclear cells. Many observers claim to have been able to produce anti-enzymes by inoculating animals with trypsin, pancreas, &c. The results are very contradictory and have to be discarded because of the action of the precipitins provoked against the proteins in the antigens used. Delezenne and Pozerski [16] state that the antitrypsin exerts its effect chiefly by combining with the kinase activating trypsinogen. Bayliss and Starling [17] oppose this view and believe that the antitrypsin acts directly upon the trypsin.

(2) Rosenthal [18] also suggested that the inhibition was due to the presence of proteoclastic cleavage bodies in the sera used. Bayliss [19], Abderhalden and Gigon [20] found that some of the products of tryptic digestion inhibited the further action of the enzyme to some extent. Later Walters [21] showed that this occurs only to a slight extent and

that the inhibition by serum is far too great to be accounted for in this way.

(3) That the antitrypsin is lipoidal in nature.

Schwarz [22] and Sugimoto [23] believe that the antitryptic action is closely related to the lipoids in the serum. They found that by treating sera with ether, benzol, olive oil, &c., the antitryptic power of the serum was greatly diminished or lost. Delezenne and Pozerski [16] found that shaking sera with chloroform destroyed their antitryptic properties. Later Jobling and Petersen [24] and others, observed the same and came to the conclusion that the antitryptic power was due to the serum lipoids and that the power could be restored by saponifying the lipoid extracted with chloroform, &c., and adding this soap to the extracted de-antitrypsinized serum. They further believed that the antitryptic power of the soap is proportional to the degree of desaturation of the fatty acid in the soap.

Cobliner [25] and Mayer [13] showed that extraction of dried sera with chloroform, ether and petroleum ether did not interfere with the antitryptic power. Jobling and Petersen [24] explain this by saying that the extraction is more effective when carried out with wet than with dried sera.

THE METHODS ADOPTED FOR DETERMINING THE ANTITRYPTIC POWER OF A SERUM.

(*a*) It was found after many experiments that the following rapid method gave results closely comparable to the much longer process detailed below, and in addition allowed of expressing a "unit"—a quantity of serum capable of inhibiting the liquefaction of a standard quantity of gelatine by a standard of trypsin.

The unit of trypsin was determined by finding the smallest amount of a 1 per cent. suspension of trypsin, which caused complete liquefaction of 1 c.c. of 15 per cent. gelatine at 37° C. in thirty minutes, all the reagents being at 37° C. before mixing. After incubation, the tubes containing the mixtures were cooled at 10° C. for thirty minutes and the amount of trypsin causing complete liquefaction determined.

The antitryptic titre of a serum was determined by adding graded quantities of the serum (usually diluted 1 in 5) to a series of tubes containing the unit of trypsin, incubating at 37° C. for thirty minutes, then adding 1 c.c. of 15 per cent. gelatine at 37° C. and incubating for thirty minutes longer at 37° C., cooling as above, and reading the unit as the smallest amount completely interfering with the liquefying action of the unit of trypsin.

(*b*) Graded quantities of the serum were added to 1 c.c. of a 1 per cent. trypsin suspension, and made up with sterile normal saline to 2 c.c. and kept for thirty minutes at 37° C., then 3 c.c. of a 2 per cent. solution of casein in 0·2 per cent. sodium carbonate were added, and the whole incubated for three hours.

The controls were (*a*) casein and trypsin incubated without serum, and (*b*) casein and trypsin without incubation made up to 5 c.c.

All the tubes were after incubation brought rapidly to the boiling point. A measured quantity was taken from each tube (2 c.c.) mixed with 15 c.c. of colloidal iron, 10 c.c. of water (N free), and 1 c.c. of sodium sulphate solution, to precipitate the coagulable nitrogen. The mixture was filtered through two wet filter papers, the filtrate was collected. A measured portion of the filtrate was taken, concentrated and then decarbonized with pure sulphuric acid. The nitrogen content was then determined by Nesslerization. The results were then plotted as curves.

This method of estimating non-coagulable nitrogen was recommended by Dr. Plimmer, and we found that it gave results identical with the other methods used, but proved more rapid and far simpler.

(I) The Nature and Properties of Antitrypsin.

(*A*) *The Antitrypsin of Egg White.*

Our first experiments were made with the white and yolk of eggs, and from them it will be seen that egg white has very marked antitryptic properties, whereas the yolk, in spite of its great lipoid content, is not antitryptic in the slightest degree. The suspension of egg white was made by dissolving up 1 grm. of vacuum dried egg albumen in 100 c.c. of normal saline. The yolk suspension was made from a fresh egg and was used in a dilution of 1 in 10 in normal saline and pure.

Experiment 1.—The egg white suspension was added to the unit of trypsin in graded quantities, starting with 10 c.mm. of the 1 in 100 dilution. The mixture was then incubated in the water bath at 37° C., for thirty minutes, when the gelatine was added as above.

10	20, 30, 40, 50, 60, 80, 100 c.mm. of 1 per cent. egg white
Almost complete inhibition of liquefaction.	Complete inhibition of liquefaction.

With the egg yolk in similar fashion:—

1 in 10.		Pure.
10, 20, 40, 60, 80, 100, 200, 400 c.mm.	...	60, 80, 100, 200 c.mm.

Complete liquefaction—i.e., no inhibition exercised by the egg yolk.

Several other experiments gave identical results. Thus egg yolk contains no antitrypsin in spite of its great lipoid content, whereas egg white, which has relatively little lipoid in its constitution, is distinctly antitryptic.

Experiment 2.—Finely powdered vacuum dried egg white was extracted with various lipoid solvents. To ensure complete dryness the powdered egg albumen was dried over sulphuric acid *in vacuo*. After extraction the extracted egg albumen was dissolved in normal saline, the solvent having been previously completely removed by decantation and drying *in vacuo*. 0·2 grm. of egg albumen was extracted in each case and the residue then taken up in 20 c.c. of normal saline.

		Complete inhibition of unit of trypsin by
(*a*) Control; unextracted ..	0·2 grm. in 20 c.c. of normal saline; then diluted 1 : 5 ...	60 c.mm. = 12 c.mm. (undiluted)
(*b*) Treated for twenty-four hours with perfectly dry ether ...	ditto ditto ...	60 c.mm. = 12 c.mm. (undiluted)
(*c*) Treated for twenty-four hours with petroleum ether ...	ditto ditto ...	60 c.mm. = 12 c.mm. (undiluted)
(*d*) Treated for twenty-four hours with absolute alcohol and then with absolute ether ...	ditto ditto ...	60 c.mm. = 12 c.mm. (undiluted)

Experiment 3.—Similar to Experiment 2.

	Complete inhibition of tryptic unit by
(*a*) Control; 1 per cent. diluted 1 : 5 ...	80 c.mm. = 16 pure; almost complete by 14 pure.
(*b*) Extracted twenty-four hours with dry ether ...	80 c.mm. = 16 pure.
(*c*) Extracted twenty-four hours with petroleum ether ...	80 c.mm. = 16 pure.
(*d*) Extracted twenty-four hours with absolute alcohol, then ether ...	90 c.mm. = 18 pure; almost complete by 16 pure.

Other experiments yielded similar results, except that occasionally, owing to imperfect drying, the ether or alcohol and ether caused some degree of coagulation of the egg albumen with corresponding loss in the antitryptic power. These experiments show that the removal of lipoids from dried egg albumen does not affect the antitryptic power of the egg white, unless the reagent has caused some degree of coagulation.

Similar treatment of a suspension of egg albumen leads to a very different result, the antitryptic power becomes completely lost. All the lipoid solvents used on a suspension of egg albumen cause a certain degree of coagulation of the protein.

Experiment 4.—A 1 per cent. suspension of vacuum dried egg albumen in normal saline exposed to different lipoidal solvents for three hours at 37° C. The egg suspension and solvent were thoroughly shaken at intervals. The solvent was removed as far as possible by decantation, the residual solvent was finally removed by exposure *in vacuo*.

	Unit of trypsin inhibited by
(*a*) Control	20 c.mm. of the suspension
(*b*) After exposure to ether	500 c.mm. ,, ,,
(*c*) ,, ,, chloroform	not inhibited by 500 c.mm.

The same result was obtained by leaving the mixture at the room temperature for three hours.

Experiment 5.—Same as Experiment 4, only kept at room temperature for three hours.

	Unit of trypsin inhibited by
(*a*) Control	20 c.mm. of the suspension
(*b*) After exposure to ether	400 c.mm. ,, ,,
(*c*) ,, ,, chloroform	not inhibited by 500 c.mm.

Other experiments gave similar results.

Jobling [24] and his co-workers obtained results similar to those of Experiment 4. They concluded that the difference between the results of the action of the lipoid solvent on a watery suspension and the dried powder *re* antitryptic destruction was due to the lipoid solvent being able to break down the lipoid-protein combination in the suspension but not in the dried state, and thus more effectually remove from the suspension the lipoids to which they ascribe the antitryptic power of a serum. They took no notice of the coagulation of the protein which occurs constantly as the result of the action of the lipoid solvent on the watery suspension, and which will destroy the antitryptic power if it occurs even when the egg white has been dried.

Experiment 6: Isolation of Antitrypsin from Egg White.—In this experiment it was endeavoured to extract antitrypsin from carefully vacuum-dried egg white. It was found later that with different commercial specimens of dried egg white the experiment was not always successful, and that this depended on the method of drying adopted.

Hamill [9] showed that the antitrypsin could be extracted from intestinal worms by treatment with 50 per cent. alcohol. We accordingly took vacuum-dried egg white, ground it up in a coffee-mill, and added two volumes of 50 per cent. alcohol, and kept the mixture at 56° C. for twenty-four hours. The supernatant alcohol was then decanted, and the residue squeezed out in a press. The fluids were mixed and filtered. A clear yellowish fluid was then obtained. This was evaporated down at 56° C., and yielded a yellowish resinous-looking residue. It was purified by adding 50 per cent. alcohol, which caused the greater part to go into solution. The solution was filtered, re-evaporated at 56° C., and again treated with 50 per cent. alcohol, and the process repeated several times. Thus the original alcoholic extract was freed from coagulable protein. This purified extract was easily soluble in normal saline, and 20 c.mm. of a 0·1 per cent. solution caused complete inhibition of the unit of trypsin as determined above. This antitryptic material was also easily soluble in distilled water. It was soluble in alcohol up to 60 per cent., above this it was precipitated, but immediately went into solution on dilution. It had the following properties: It was free from coagulable protein; it gave a xanthoproteic reaction with nitric acid, also a rose-red biuret reaction with copper sulphate and caustic soda; it was precipitated by 50 per cent. ammonium sulphate; its antitryptic properties were in no way affected by treatment with lipoidal solvents such as chloroform, ether, alcohol; it was non-dialysable, on dialysis some salt and carbohydrate diffused away, but the antitryptic power was not impaired. The purified fat sugar and salt free antitrypsin was therefore a colloid and had protein characters.

Experiment 7: Action of various Re-agents on Prepared Antitrypsin.—A 0·1 per cent. suspension in normal saline was used in all the tests in this experiment.

(*a*) *Action of Lactic Acid.*

		Made up to			
	Control	n/25	n/20	n/15	n/10
The unit was contained in	20	20	20	20	25 c.mm. of the suspension

(*b*) *Boiling with Acid and Alkali.*

	Control	Control boiled 15 minutes in water bath	Made up to n/20 lactic acid and boiled as before	Made up to n/20 alkali and treated as before
Unit contained in	15	20	20	Not in 200 c.mm.

(*c*) *Adsorption.*—Quantities of 5 c.mm. of the antitryptic suspension were exposed to the various reagents for two hours at 37° C.; the mixtures were periodically shaken. The agar was added in the solid state and then cooled to gelatine before incubation. The mixtures were centrifuged; correction made for dilution (uncorrected figures in brackets).

	Control	200 mg. kaolin	n/20 lactic acid	1 c.c. of 0·25 per cent. starch	0·5 c.c. of 0·5 per cent. agar	*Bacillus coli*	200 mg. blood charcoal
Unit in	20 c.mm.	20 c.mm.	(25 c.mm.) =21 c.mm.	(25 c.mm.) =21 c.mm.	(25 c.mm.) =22 c.mm.	20 c.mm.	20 c.mm.

Exposure to the same re-agents in the cold gave similar results.

From these experiments we conclude that:—

(1) The antitryptic power of egg albumen is not due to lipoids because: (*a*) Egg white does not lose its antitryptic properties when treated with lipoid solvents unless they cause more or less coagulation of the protein; (*b*) prepared dry antitrypsin is unaffected by lipoid solvents; (*c*) egg yolk which is rich in lipoids is absolutely non-antitryptic.

(2) The antitryptic power appears to be due to a body which is (*a*) a non-diffusible protein substance; (*b*) not destroyed by boiling or by the action of dilute acids; (*c*) destroyed by weak alkalis; (*d*) soluble in 60 per cent. alcohol but is precipitated by alcohols of higher concentration, but not coagulated, going into solution directly the concentration is lowered; (*e*) precipitated by 50 per cent. ammonium sulphate; (*f*) not absorbed or adsorbed by charcoal, kaolin, &c., agar starch or bacteria; (*g*) not affected by treatment in the dry state with lipoid solvents.

(*B*) *Antitrypsin of Blood Serum.*

Having thus isolated the antitrypsin from egg, similar means were adopted to endeavour to isolate it from blood serum.

Experiment 8.—For this purpose the serum was dried *in vacuo* over sulphuric acid. This did not affect its subsequent complete solubility. The dried serum was then treated with 50 per cent. alcohol, but it was impossible to extract an antitryptic substance by this method. Similar experiments were made with concentrations of alcohol varying from 10 to 80 per cent. but without any success. The direct method of extraction having failed we next worked by exclusion, investigating the results of the extractions of the lipoids from the sera experimented with, and also the antitryptic effects of these lipoids and their soaps.

Experiment 9.—Treatment of sera with various lipoidal solvents in the wet state destroys the antitryptic power of the serum. (*a*) 20 c.c. of rabbit serum were repeatedly thoroughly shaken up with 40 c.c. of chloroform and kept at 37° C. for one hour. At the end of the time the mixture was centrifuged and the supernatant clear serum withdrawn, and all traces of chloroform removed by exposure *in vacuo*. The deposit was a thick one consisting of chloroform and coagulated protein. The original titre of the serum by the gelatine method was 60 c.mm. After chloroform extraction the serum did not contain this unit in 500 c.mm. Similar experiments gave the same results: destruction of the antitryptic power. (*b*) Ether: 20 c.c. of rabbit serum were thoroughly repeatedly shaken with 40 c.c. of ether and kept at 37° C. for three hours (renewing the ether periodically). At the end of the time there were three layers—a bottom of coagulated protein, a middle of clear serum and a top of ether. The latter was removed, the clear serum pipetted off and exposed *in vacuo* to remove the ether.

Titre before ......60 c.mm. inhibited the unit of trypsin.
Titre after ......500 c.mm. did not inhibit the unit of trypsin.

Experiment 10.—Same experiment as Experiment 9, only the mixture was kept at the room temperature. (*a*) 20 c.c. of rabbit serum were repeatedly shaken up in 40 c.c. of chloroform and kept at the room temperature for three hours. At the end of the time there was a heavy deposit consisting of chloroform emulsion and coagulated albumen and a somewhat turbid upper layer. The latter was pipetted off, the chloroform removed *in vacuo* and the bulk restored by the addition of normal saline. Before treatment, 60 c.mm. inhibited the unit of trypsin. After treatment, 500 c.mm. did not inhibit the unit of trypsin. (*b*) Serum similarly treated with ether. After treatment 500 c.mm. did not inhibit the unit of trypsin. Hence we must conclude that shaking and extracting liquid sera with lipoidal solvents such as chloroform or ether destroys the antitryptic power of the serum, but it must be remembered that these reagents not only dissolve out some of the lipoids but also profoundly alter the proteins, precipitating and coagulating a portion of them. These experiments also show that the antitryptic loss is not proportional to the amount of lipoid extracted since the loss of power is about the same if the extraction be carried on at 37° C. or at room temperature.

Experiment 11: Extracting Dried Sera with various Lipoid Solvents.—Carefully measured quantities of sera were evaporated in vacuo in very thin layers in shallow dishes. The dried serum was finely powdered and again dried *in vacuo*. Extraction was then performed. After extraction the solvent was carefully filtered off through a small very fine filter paper. The filter paper and residue were dried *in vacuo* and then treated with normal saline. (*a*) 5 c.c. of guinea-pig's serum was used in each test. After extraction the dried residue was made up to 5 c.c. with normal saline.

	Unit of trypsin inhibited by
Control, not evaporated	40 cmm.
,, dried and re-dissolved	40 ,,
Serum dried, extracted with chloroform at 37° C. for three hours	40 ,,
,, ,, ,, ether at 37° C. for three hours...	40 ,,
,, ,, ,, petroleum ether at 37° C. for three hours	40 ,,

(*b*) Rabbit serum: 5 c.c. used in each test. After extraction the total residue was dissolved in 10 c.c. of normal saline.

	Unit of trypsin inhibited by
Control; dried only	100 c.mm. = 50 c.mm. pure
Extracted with chloroform	100 ,, = 50 ,, ,,
Extracted with petroleum ether	120 ,, (almost C. I. with 100 c.mm.) = 60 c.mm. pure
Extracted with xylol	100 c.mm. = 50 c.mm. pure

(*c*) Rabbit serum: Quantities as (*b*) only made up to original bulk.

	Unit of trypsin inhibited by
Control; dried	30 c.mm. of serum
Extracted with absolute alcohol at 37° C. for six hours	30 ,, ,,
Extracted with absolute alcohol for three hours, then chloroform for four hours at 37° C.	30 ,, ,,

(*d*) Rabbit serum: 10 c.c. in each test. Carefully dried as above. Residues taken up in 50 c.c. of normal saline.

	Unit of trypsin inhibited by
Control; dried only	48 c.mm. pure
Extracted with absolute alcohol for twenty-four hours, then with chloroform for six hours; after evaporation of the solvent it was found that the solution in the solvent was very turbid	titre 72 c.mm. pure

So that in spite of some coagulation of the protein the antitryptic power was 66 per cent. of the control.

(*e*) Rabbit serum dried *in vacuo* in quantities of 2 c.c. in very large plates so as to get a very thin film of dried serum.

	Unit of trypsin inhibited by
(1) Control dissolved in 10 c.c. of saline	160 c.mm. = 32 pure
(2) Treated with absolute alcohol sealed up for twenty-four hours, alcohol evaporated, then chloroform for four hours, all at 37° C.	160 c.mm. = 32 pure
(3) Treated with alcohol alone for twenty-four hours at 37° C.	160 c.mm. = 32 pure

(*f*) Same as (*e*) only taken up in 5 c.c. of normal saline.

	Unit of trypsin inhibited by
(1) Control : dried serum	70 c.mm. = 28 pure
(2) Extracted with absolute alcohol for twenty-four hours at 37° C.	70 c.mm. = 28 pure
(3) Extracted with alcohol for twenty-four hours, then chloroform for six hours at 37° C.	80 c.mm. = 32 pure
(4) Chloroform extraction only, twenty-four hours ...	70 c.mm. = 28 pure

These experiments undoubtedly show that lipoidal solvents at 37° C. do not remove the antitryptic power from dried sera. Further, some of the methods used, alcohol followed by chloroform extraction, not only dissolve out the uncombined lipoids but also the bound lipoids. This disposes of the objection put forward by Jobling [24] to explain the difference between the results obtained when fresh and dried sera are extracted. Jobling states that in the wet state the combined lipoids are split off from their combination by chloroform or ether and so extraction in this state leads to a greater removal of lipoids than when the serum is dried. Besides being contrary to usually accepted facts the following experiment shows the assumption to be wrong.

Experiment 12.—Equal quantities of ox serum were taken. (1) One portion was dried *in vacuo*, ground up finely, extracted with chloroform at 37° C. for twenty-four hours. (2) The other was repeatedly shaken up with 2 vols. of chloroform and kept at 37° C. for twenty-four hours. The mixture was centrifuged, the deposit taken up—treated with absolute alcohol and ether to clear and precipitate the albumen. The extracts (1) and (2) were then evaporated and boiled with 20 c.mm. of N/10 alcoholic potash, and excess of alkali determined.

(1) 20 — 3·5 c.c. = 16·5 c.c. N/10 alkali were required to saponify the fat extracted.
(2) 20 — 8·0 c.c. = 12·0 c.c. N/10 alkali were required to saponify the fat extracted.

Showing that more fats are dissolved out by chloroform extraction of dried serum than fresh serum.

Finally the method of Cathcart [26] for completely removing the lipoids from a serum was carried out.

Experiment 13.—(*a*) 5 c.c. of guinea-pig's serum were cooled to 0° C., then poured into 40 c.c. of absolute alcohol at −14° C. and left for one hour, filtered in cold filter at the same temperature, washed with absolute dry ether at −8° C., then extracted with boiling dry ether for twenty-four hours.

Titre of serum (50 per cent.)	50 c.mm. = 25 pure
Titre of extracted serum (50 per cent.)	50 c.mm. = 25 pure

(In spite of small loss of material during the process.)

(*b*) Similar experiment to last.

Titre before ... 30 c.mm. | Titre after ... 70 c.mm.
(But here there was great loss of precipitate during transference to filter paper and there was some coagulation.)

(*c*) Similar experiment.

Control ... ... 20 c.mm. | After extraction ... 25 c.mm.
(Here again there was loss of material due to manipulation.)

(*d*) 5 c.c. of rabbit serum was treated as above. The residue was taken up in 10 c.c. of normal saline.

Titre before extraction (50 per cent.) = 100 c.mm. = 50 pure.
Titre after extraction (50 per cent.) = 100 c.mm. = 50 pure.

(*e*) 10 c.c. of guinea-pig's serum was treated as above, very carefully; there was very little loss of precipitate during the experiment and the precipitate after complete extraction readily went into solution. The serum and precipitate were made up to 50 c.c.

Before treatment the serum inhibited unit of trypsin in 120 c.mm. = 24 pure.
After treatment the precipitate inhibited unit of trypsin in 120 c.mm. = 24 pure.

Hence although we have not succeeded in isolating serum antitrypsin we have been able definitely to prove that it is not lipoidal in nature contrary to the results of nearly all previous workers. Complete removal of the lipoids without interfering with the solubility of the protein in no way affects the antitryptic titre. Hence we must conclude that the antitryptic power of the serum is not due either to the free or combined lipoids.[1]

(*C*) *Soaps as Antitryptic Agents.*

Jobling [24] in support of his view that the serum lipoids are antitryptic shows that the extracted lipoids when saponified are antitryptic. He further states that the antitryptic power of a soap depends upon the degree of desaturation of its fatty acids. Our experiments go to prove that all soaps destroy trypsin, no matter how unsaturated the fatty acids are. Their action is quite different from that of true antitrypsin. As will be seen later there is not much difference in the amount of inhibition exercised by a given quantity of serum if the experiment is performed in two stages or in one, viz., if the serum is allowed to act on the trypsin for some time before the gelatine is added or if all three are added simultaneously. In the case of the soaps there is a great difference in the amounts necessary to produce inhibition if the experiment is performed in two stages or one, i.e., the soaps depend for their antitryptic action on the degree of concentration.

Experiment 14.—Suspensions of the following soaps were prepared and the amounts necessary to inhibit the unit of trypsin were determined: (*a*) with the soap, trypsin and gelatine incubated simultaneously; (*b*) with the soap and trypsin incubated together for thirty minutes at 37° C. first, and then the gelatine added.

	(*a*)	(*b*)	Iodine number of soap
5 per cent. sodium oleate	400 c.mm. inhibit unit of trypsin	30 c.mm. inhibit unit of trypsin	86
5 per cent. sodium linoleate soap	500 c.mm. inhibit unit of trypsin	50 c.mm. inhibit unit of trypsin	176
5 per cent. sodium stearate soap	500 c.mm. do not inhibit unit of trypsin	10 c.mm. inhibit unit of trypsin	78
2½ per cent. sodium stearate soap	500 c.mm. do not inhibit unit of trypsin	20 c.mm. inhibit unit of trypsin	78
5 per cent. croton oil soap	500 c.mm. inhibit unit of trypsin	10 c.mm. inhibit unit of trypsin	104
5 per cent. sodium linoleate soap (another specimen)	Ditto	30 c.mm. inhibit unit of trypsin	188
Ditto, completely saturated with iodine (none free)	Ditto	Ditto	*Nil*
2½ per cent. soap made from chloroform extract of dried ox serum	Ditto	20 c.mm. inhibit unit of trypsin	—

These soaps were all prepared by Mr. Reeve, B.Sc., of the Department of Physiological Chemistry, University College.

In connexion with this the following experiment is interesting in bringing out the difference between the mode of action of antitrypsin and soap.

Experiment 15.—(*a*) The different constituents in the experiment were incubated together; (*b*) The antitrypsin and trypsin were incubated together first for thirty minutes at 37° C., then the gelatine added.

	(*a*)	(*b*)
(1) Serum; unit of trypsin inhibited by	20 c.mm. ...	16 c.mm.
(2) ,, ,, ,, ,, ...	20 ,, ...	16 ,,
(Very marked inhibition by 16 c.mm.)		
(3) 5 per cent. sodium oleate (iodine No. 86); unit of trypsin inhibited by	100 ,, ...	10 ,,
(4) 5 per cent. sodium linoleate soap; unit of trypsin inhibited by	300 ,, ...	20 ,,

These experiments are at variance with those of Jobling [24] and show: (1) that the soaps act quite differently from the serum antitrypsin—the effect of concentration of the former is very apparent, in the latter this is not apparent to any marked degree; (2) that the desaturation of the fatty acids in the soap makes no appreciable difference; (3) that the soaps from the uncombined lipoids act quite well antitryptically—and according to Jobling it is the combined lipoid which is antitryptic; (4) that the soaps destroy trypsin in virtue of the hydroxyl-ion concentration.

The following experiment, using the casein digestion method, brings out the slight difference in the action of antitrypsin if allowed to act alone in the trypsin first or if all constituents are added together.

Experiment 16.

	50 c.mm serum	100 c.mm. serum
(*A*) Control N.C.N. determined trypsin and casein and serum; no incubation	1·9 mgr.	1·95 mgr. of N.
(*B*) Serum—trypsin and casein incubated together for three hours at 37° C.	4·4 mgr.	4·3 mgr.
(*C*) Serum — trypsin incubated for thirty minutes at 37° C., then casein added and incubated for three hours at 37° C.	3·9 mgr.	3·9 mgr.

(1 c.c. of 1 per cent. trypsin, 3 c.c. of 2 per cent. solution of casein used in each case. All made up to 5 c mm.)

The separate incubation makes a difference of about 13 per cent., thus agreeing with the gelatine experiments.

(D and E).—*Inhibition of Antitrypsin, Adsorption of Antitrypsin in Serum.*

We have previously shown that lipoidal solvents destroy the antitrypsin in fluid sera, but find that this is due to the precipitation of proteins, and not due to the removal of the fats The following experiments show the effect of acids and alkalis on serum antitrypsin. It was, however, first necessary to find the limits of acids or alkali concentration interfering with tryptic action. We accordingly exposed the trypsin to acids and alkalis of various strengths, and tested the result with gelatine liquefaction.

Experiment 17.—(*a*) 200 c.mm. of 1 per cent. trypsin (the unit causing complete liquefaction of 1 c.c. of 15 per cent. gelatine in thirty minutes at 37° C.), and 1 c.c. of gelatine were put into a series of tubes and the reaction was adjusted as follows. Incubation for thirty minutes at 37° C. was allowed. Then readings taken as usual.

	n/10	n/20	n/50	n/100	n/120	n/200	n/250	n/500
Acid ...	CL	ACI	CL	CL	CL	CL	CL	CL
Alkali ..	SI	CL	CL	CL	CL	CL	CL	CL

Thus n/20 acid interferes distinctly with trypsin.
n/10 alkali only to a slight degree.

(*b*) As before, a mixture of gelatine and 1 per cent. trypsin was made in the proportion of 200 c.mm. of the latter to 1 c.c. of the former. The reaction was then adjusted as below.

	n/5	n/7	n/10	n/11	n/13	n/14	n/16	n/20	n/25	n/33	n/50	n/100
Lactic acid	CI	CI	CI	CI	CI	CI	CI	CI	SI	CL	CL	CL
Alkali ...	CL	CL	CI	ACI	SI	CL	CL	CL	CL	CL	CL	CL

CI = Complete inhibition
ACI = Almost complete inhibition
SI = Slight inhibition
CL = Complete liquefaction
} from action of alkali.

Experiment 18 : Action of Lactic Acid on Serum Antitrypsin.—(1) 2 c.c. of rabbit serum in each test incubated for three hours at 37° C., with acid as follows: then the antitryptic titre was taken as before.

Reaction	n/66	n/50	n/40	n/30	n/30	n/28	n/22	n/20	n/10 acid
Amount of serum inhibiting unit of trypsin ...	50	50	60	60	60	70	200	250	300 c.mm.

(2) Similar experiment. Lactic acid, three hours' incubation at 37° C. as above.

Reaction	n/100	n/80	n/60	n/60	n/20
Amount of serum inhibiting the unit of trypsin ...	60	60	60	60	300 c.mm.

Experiment 19 : Rate of Destruction of Antitrypsin by Lactic Acid.—From Experiment 18 it was seen that n/20 lactic acid concentration destroyed antitrypsin in the serum. The following experiments show rate of destruction.

	Serum made up to n/20 Lactic Acid.						
	Control	Immediately after acidification	Five minutes later*	Fifteen minutes later*	Twenty minutes later*	Twenty-five minutes later*	Thirty-five minutes later*
Quantity of serum inhibiting unit of trypsin	70	90	150	150	250	300	500 c.mm.

* In water bath at 37° C.

Experiment 20: Action of Alkali.—Rabbit serum made up to the following concentration NaOH incubated for three hours at 37° C., then tested. (1)

	n/100	n/80	n/60	n/40	n/20	Control	
Amount of serum inhibiting unit of trypsin	60	60	60	60	70	60	c.mm.

(2) Another specimen:—

Alkali		n/100	n/50	n/20	Control	
Three hours at 37° C.	...	80	80	200	80	c.mm.
Twenty hours in cold	...	80	80	200	80	,,

Experiment 21: The Titre cannot be brought back on Neutralization.—(1) Rabbit serum made up to n/20 lactic acid, incubated for forty-five minutes in water bath at 37° C., then neutralized with N/1 NaOH.

	Control	After ¾ hour's incubation in n/20 acid	After neutralization
Amount of serum inhibiting unit of trypsin	60	400	400 c.mm.

A deposit was formed on neutralization. This was taken up and was found to have no antitryptic power.

(2) Rabbit serum was made alkaline with NaOH to the desired degree, incubated for twenty minutes in the water bath at 37° C., then neutralized.

Amouut of serum inhibiting the unit of trypsin—

		Control	n/20	n/10	n/5	
Before neutralization	..	40	80	200	500	c.mm.
After ,, ...	...	—	80	200	500	,,

Experiment 22: Effect of Temperature on Antitrypsin.—(1) Rabbit serum incubated in the water bath at:—

Control			Amount of serum inhibiting the unit of trypsin 56° C.		60° C.
0 minutes	...	...	40 c.mm.	...	40 c.mm.
15 ,,	..	..	40 ,,	...	80 ,,
30 ,,	...	...	50 ,,	...	100 ,,
60 ,,	...	...	60 ,,	...	160 ,,
90 ,,	...	...	60 ,,	...	200
180 ,,	...	...	80 ,,	...	400 ,

(2) Another experiment. Rabbit serum kept at—

Control			Amount inhibiting the unit of trypsin 56° C.		60° C.
0 minutes	...	...	30 c.mm.	...	30 c.mm.
30 ,,	...	...	30 ,,	...	120 ,,
60 ,,	...	...	30 ,,	...	120 ,,
2 hours	...	...	30 ,,	...	300 ,,
3 ,,	...	...	30 ,,	...	300 ,,
4 ,,	...	...	30 ,,	...	400 ,,

Experiment 23 : Effect of Incubation on Antitrypsin in Bacteriologically Sterile Serum.—(1) Sterile rabbit serum.

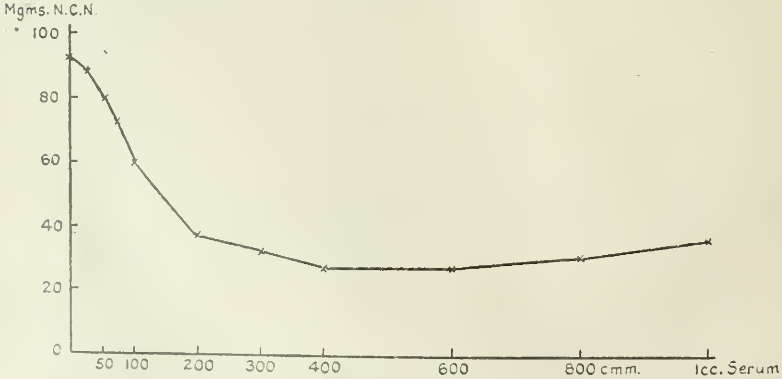
	Control	72 hours	120 hours
Unit of trypsin inhibited by	30	30	30 c.mm. of serum

(2) Dog's serum.

	Control	4 hours' incubation	24 hours	14 days
Unit of serum inhibited by	60	50	50	50 c.mm. of serum

Experiment 24.—During the course of the previous experiments, where the casein method was used as a control, it came out that the amount of inhibition by serum antitrypsin was not proportional to the amount used, and even when apparently a large excess had been used some digestion still occurred. Thus in the following experiment graded quantities of guinea-pig serum (diluted 1 in 5, and pure as required) were added to 1 c.c. of 1 per cent. trypsin, and all the tubes made up to 2 c.c. with normal saline incubated at 37° C. for thirty minutes, then 3 c.c. of a 2 per cent. casein solution added ; digestion two hours at 37° C.

Amount of serum	Non-coagulable nitrogen
0 c.c.	= 92 mgr. per cent.
0·025 ,,	= 88 ,, ,,
0·05 ,,	= 80 ,, ,,
0·075 ,,	= 72 ,, ,,
0·1 ,,	= 60 ,, ,,
0·2 ,,	= 38 ,, ,,
0·3 ,,	= 32 ,, ,,
0·4 ,,	= 28 ,, ,,
0·6 ,,	= 28 ,, ,,
0·8 ,,	= 30 ,, ,,
1 ,,	= 32 ,, ,,
Control trypsin and casein, no incubation	= 24 ,, ,,



Experiment 24.—To show inhibition by varying amounts of antitrypsin.

(G) *Experiment 25: The Protein Fraction of the Serum containing the Antitrypsin.*—Serum treated with $Am_2 SO_4$.

	Titre	True
(1) 33 per cent. saturation to precipitate euglobulin—euglobulin solution	600 c.mm.	300 c.mm.
(2) 50 per cent. saturation to precipitate euglobulin and pseudoglobulin, solution of the two	300 ,,	150 ,,
(3) Solution of pseudoglobulin and albumen from (1), after precipitation of euglobulin	100 ,,	50 ,,
(4) Solution of albumen from (2)	240 ,,	120 ,,
(5) Control, diluted 50 per cent.	80 ,,	40 ,,

All made up to double the volume of the original serum.

Experiment 26.—Similar experiment.

	Titre	True titre
(1) Control	50 c.mm.	25 c.mm.
(2) Control + equal amount of saturated ammonium sulphate	100 ,,	25 ,,
(3) Euglobulin precipitate with 33 per cent. ammonium sulphate	not 400 ,,	— ,,
(4) Albumen after precipitation of rest with 50 per cent. ammonium sulphate	200 ,,	100 ,,
(5) Euglobulin + pseudoglobulin preparation from (4)	300 ,,	150 ,,
(6) Albumen + pseudoglobulin, after (3)	90 ,,	45 ,,

Hence the euglobulin fraction is not antitryptic, but the pseudoglobulin and albumen fractions are. These experiments are in agreement with those of Cathcart [10] who stated that the albumen fraction was antitryptic.

(II) AUTOLYSIS OF SERUM.

All the sera used for the following experiments were collected with the necessary aseptic precautions. Sera showing evidence of contamination at any stage of the experiment were rejected. Non-coagulable nitrogen was determined as before (*see* p. 9.)

(*A*) *Incubation of Sera without Previous Destruction of the Antitrypsin.*

Experiment 1.

	Antitryptic titre	Non-coagulable nitrogen
(*a*) Rabbit serum—		
Control	60 c.mm.	32 mgr. per cent.
After four hours at 37° C.	50 ,,	32 ,, ,,
After twenty-four hours at 37° C.	50 ,,	32 ,, ,,
(*b*) Rabbit serum tinged with hæmoglobin—		
Control	40 ,,	32 ,, ,,
After seventy-two hours at 37° C.	40 ,,	34 ,, ,,
After 120 hours at 37° C.	40 ,,	40 ,, ,,

Experiment I (continued).

	Antitryptic titre	Non-coagulable nitrogen
(*c*) Dog's serum—		
Control	30 c.mm.	45 mgr. per cent.
After 120 hours at 37° C.	30 ,,	45 ,, ,,
(*d*) Dog's serum—		
Control	30 ,,	52·5 ,, ,,
Seventy-two hours at 37° C.	30 ,,	52·5 ,, ,,
(*e*) Sheep's serum—		
Control	60 ,,	32 ,, ,,
Seventy-two hours at 37° C.	60 ,,	32 ,, ,,
Fourteen days at 37° C.	50 ,,	32 ,, ,,
(*f*) Sheep's serum, tinged with hæmoglobin—		
Control	50 ,,	40 ,, ,,
Fourteen days at 37° C.	50 ,,	52 ,, ,,
(*g*) Sterile guinea-pig's serum—		
Control	20 ,,	34 ,, ,,
Twenty-four hours at 37° C.	20 ,,	34 ,, ,,
Seventy-two hours at 37° C.	20 ,,	34 ,, ,,

From these experiments it is obvious that the serum antitrypsin inhibits any proteolytic autolytic ferment in the serum. It, however, does not interfere with the autolytic ferment in the red cells. Antitrypsin is therefore also not destroyed by the proteolytic ferment as it is by trypsin.

(*B*) *Effect of Destruction of the Antitrypsin.*

Experiment 2.—(*a*) Sterile rabbit serum was made N/20 lactic acid by adding the requisite amount of n/1 lactic acid, then incubated.

	Antitryptic titre	Non-coagulable nitrogen
Control, before lactic acid	60 c.mm.	32 mgr. per cent.
Eight days at 37° C., after lactic acid	not in 500 c.mm.	32 ,, ,,
Twelve ,, ,, ,, ,,	,, ,,	32 ,, ,,

(*b*) Sterile rabbit serum: Destruction of antitrypsin by heat and acid.

	Antitryptic titre	Non-coagulable nitrogen 24 hours	Non-coagulable nitrogen 72 hours
Control	60 c.mm.	36	— mgr. per cent.
Made n/20 lactic acid	not 500 c.mm	36	36 ,, ,,
Inactivated at 60° C.	,, ,,	36	36 ,, ,,
Inactivated and made n/20 lactic acid	,, ,,	36	36 ,, ,,

(*c*) Sterile dog's serum.

	Non-coagulable nitrogen
Control	45 mgr. per cent.
Incubated five days	45 ,, ,,
Made n/20 acid and incubated five days	45 ,, ,,

(*d*) Sterile guinea-pig serum.

	Antitryptic titre	Incubated 24 hours	Incubated 96 hours
		Non-coagulable nitrogen	
Control	20 c.mm.	32	— mgr. per cent.
Made n/20 lactic acid	not 500 c.mm.	32	32 ,, ,,
Inactivated at 60° C. for forty-five minutes	,, ,,	32	32 ,, ,,

Hence after destroying the antitrypsin by (*a*) lactic acid, (*b*) heating, there is no evidence of autodigestion.

Experiment 3.—(*a*) Sterile rabbit serum was heated at 37° C. with chloroform as for destruction of antitrypsin. The chloroform was removed and the serum evaporated *in vacuo* sterilely to remove last traces of chloroform, made up to original bulk with normal saline.

	Antitryptic titre	Non-coagulable nitrogen
Control	not in 500 cmm.	32 mgr. per cent.
Incubated three days at 37° C.	,, ,,	,, ,,

(*b*) Similar experiment with guinea-pig serum.

	Antitryptic titre	Non-coagulable nitrogen
Control, after treatment with chloroform	300 c.mm.	34 mgr. per cent.
Incubated three days at 37° C.	300 ,,	34 ,, ,,

(*c*) Sterile sheep's serum was treated with (*a*) chloroform at 37° C., (*b*) ether at room temperature, (*c*) iodine, to destroy antitrypsin. In the case of (*a*) and (*b*) the solvent was removed, and the serum evaporated *in vacuo* to get rid of the last traces, then made up to the original volume with normal saline. Each specimen was sterile by tests before and after incubation.

	Ether serum	Chloroform serum	Iodine serum
Non-coagulable nitrogen—			
Before incubation	40	40	35 mgr. per cent.
Seventy-two hours at 37° C.	40	40	35 ,, ,,
120 hours at 37° C.	40	40	35 ,, ,,
Antitryptic titre	not in 500 c.mm.	Just in 500 c.mm.	

From these experiments it is seen that autodigestion of the serum does not occur when the antitrypsin is destroyed by (*a*) n/20 lactic acid, (*b*) chloroform at 37° C., (*c*) ether at room temperature (sheep serum), (*d*) iodine. This may be due to (*a*) absence of an autolytic ferment, (*b*) destruction of the ferments by the reagents used to destroy the antitrypsin.

(C) (1) To investigate the latter point the following experiments were performed:—

Experiment 4.—(*a*) Sterile rabbit serum was treated with: (i) Chloroform in the cold (room temperature) shaking and leaving for two hours; (ii) ether in the cold. The solvents were removed, the sera evaporated to dryness *in vacuo* to remove the solvents completely, then made up to original volume with sterile normal saline.

	Antitryptic titre	Non-coagulable Nitrogen		Increase
		Incubated 24 hours at 37° C.	Incubated 72 hours at 37° C.	
Control	60 c.mm.	34 mgr. (not incubated)	—	—
Chloroform serum	500 ,,	36 mgr.	37 mgr.	10 per cent.
Ether serum	500 ,,	34 ,,	34 ,,	—
(*b*) Sterile guinea-pig's serum, treated as in (*a*).				
Control	20 c.mm.	32 mgr.	—	—
Chloroform serum	500 ,,	38 ,,	38 mgr.	19 per cent.
Ether serum	500 ,,	36 ,,	36 ,,	12 ,,
(*c*) Sterile dog serum, treated as above.				
Control	30 c.mm.	45 mgr.	—	—
Chloroform (room temperature)	not in 500 c.mm.	52 ,,	52 mgr.	15 per cent.
Chloroform (37° C. for one hour)	,, ,,	48 ,,	48 ,,	6 ,,
Ether (room temperature)	,, ,,	48 ,,	48 ,,	6 ,,
(*d*) Human serum, treated as above.				
Control	40 c.mm.	28 mgr.	—	—
Chloroform serum (room temperature)	not in 500 c.mm.	29·5 ,,	30 mgr.	—
Ether	,, ,,	30 ,,	30 ,,	—

These reagents only act by destroying the antitrypsin and have no direct activating effect on the ferment.

Experiment 5.—Equal quantities of sterile guinea-pig serum were evaporated *in vacuo* and ground up to a fine powder and extracted with: (*a*) chloroform at room temperature, (*b*) chloroform at 37° C., (*c*) ether, (*d*) alcohol and then chloroform for twenty-four hours. The solvents were removed and the dried extracted residues were taken up in normal saline to original bulk and incubated for twenty-four hours at 37° C.

Antitryptic titre	Non-coagulable nitrogen Before incubation	After incubation
(*a*) 20 c.mm.	32 mgr.	32 mgr. per cent.
(*b*) 20 ,,	32 ,,	32 ,, ,,
(*c*) 20 ,,	32 ,,	32 ,, ,,
(*d*) 20 ,,	32 ,,	32 ,, ,,

At the same time similar portions (*e*) and (*f*) of the serum after treatment as (*a*) and (*b*) were dissolved in normal saline and treated with chloroform at room temperature and then the solvent removed in the usual way.

(*e*)	First at 37° C.	not in 500 ...	32 ...	38
(*f*)	First at room temperature ...	,, ,, ...	32 ...	38

Hence it is only the destruction of the antitrypsin which activates the serum ferment. Further, the experiment shows that the ferment is not inactivated by chloroform at 37° C. when the serum is dried.

Effect of Reaction on the Ferment.

Experiment 6.—Sterile guinea-pig's serum was treated as above with chloroform at the room temperature for the destruction of antitrypsin. After complete removal of the solvent by drying *in vacuo* the serum was dissolved in sterile saline and the reaction adjusted with standard sterile alkali and acid (N/1, N/10, N/20) to make the following concentrations and then incubated at 37° C. for twenty-four hours.

	Non-coagulable nitrogen									
	Control	n/20	n/40	n/80	n/100	n/200	n/400	n/600	n/800	
Acid (lactic) ...	32	32	32	32	32	32	34	38	38	mgr. per cent.
Alkali (NaOH) ...	32	32	32	32	32	32	32	38	39	,, ,,

From this it will be seen that the autolytic ferment is very sensitive to reaction, acid or alkali. About n/400 either acid or alkali appears to destroy the ferment, hence the reason why the ferment cannot be demonstrated when the antitrypsin is destroyed by acid or alkali; the necessary concentration n/20 is injurious to the ferment.

(*C*) (3) *Effect of Heat.*

Experiment 7.—Sterile guinea-pig's serum deantitrypsinized as above by chloroform in the cold. Heated at 56° C. and 60° C. for various periods and then incubated at 37° C. for twenty-four hours and the N.C.N. determined.

Exposure	30 minutes	60 minutes	90 minutes	120 minutes	4 hours	
At 56° C. ...	34 ...	34 ...	34 ...	34 ...	34	mgr. non-coagulable nitrogen per cent.
At 60° C. ...	34 ...	34 ...	34 ...	34 ...	34	,, ,, ,,

Control 34 mgr. non-coagulable nitrogen per cent. and incubated for twenty-four hours, 39 mgr. non-coagulable nitrogen per cent.

Hence the autolytic ferment shows the slightly greater sensitiveness to temperature than antitrypsin does.

(D) *Adsorption of the Enzyme.*

It was found that if the deantitrypsinized serum (by chloroform in the cold) was exposed to sterile blood charcoal, 0·5 per cent. suspension of agar solution, or kaolin, that digestion did not take place.

Experiment 8.—Guinea-pig's serum was deantitrypsinized by chloroform in the cold, repeatedly shaken. The chloroform was removed, the serum made up to original bulk by addition of sterile saline.

	Antitryptic titre	Non-coagulable Nitrogen	
		Before incubation	After 24 hours at 37° C.
(*a*) Serum alone	not in 500 c.mm.	32 mgr. per cent.	38 mgr. per cent.
(*b*) Exposed to blood charcoal, then centrifuged (100 mgr. to 10 c.c.)	,, ,,	32 ,, ,,	32 ,, ,,
(*c*) Exposed to agar (1 c.c. of 0·5 per cent. agar solution, 10 c.c. of serum mixed at 37°C., then iced to gelatine)	,, ,,	30 ,, ,,	30 ,, ,,
(*d*) Exposed to kaolin (200 mgr., then centrifuged to 5 c.c.)	,, ,,	32 ,, ,,	32 ,, ,,

This experiment is especially of interest and importance in conjunction with the results obtained *re* the antitryptic adsorption and the production of anaphylotoxin *in vitro* by the same substances.

Jobling's [24] experiments showed adsorption of antitrypsin, rise in N.C.N. and production of anaphylotoxin; our experiments show that although a potent "anaphylotoxin" can be obtained by exposing guinea-pig's serum to agar, &c., there is (1) no adsorption of antitrypsin by the reagent; (2) no rise in N.C.N.; (3) an adsorption of the proteolytic autolytic ferment. Hence these experiments are completely at variance with those of Jobling, and show that the "anaphylotoxin" is produced without any evidence of autodigestion and by substances which do not cause the adsorption of the antitrypsin. Hence we conclude that (1) there is a weak proteolytic autolytic ferment in the serum of the guinea-pig, less in the other animals experimented with (human, sheep, rabbit, dog); (2) the autolytic ferment is easily destroyed by (*a*) slight variation in the reaction (n/400); (*b*) the reagents which destroy antitrypsin; (*c*) and by heating (56° C.). The importance of this lies in the relation of this ferment to "anaphylotoxin" production *in vivo* and *in vitro* and the so-called protective ferments of Abderhalden [27] which will be discussed later.

The following experiment is of great importance *re* the activity of the autolytic proteolytic ferment of the serum *in vivo*.

Experiment 9.—A rabbit was inoculated subcutaneously with 1 c.c. of oleum phosphoratum B.P. Previous experiment had shown that this causes the antitryptic titre to fall almost to *nil* a few hours before the death of the animal. The animal, as already mentioned elsewhere, suffered no pain from the inoculation and continued to eat well till shortly before it was killed. The serum of this animal gave the following results.

	Non-coagulable nitrogen—		
Antitryptic titre	Unincubated	Incubated 24 hours	Incubated 4 days
500 c.mm. ...	78 mgr. per cent.	78 mgr. per cent.	78 mgr. per cent.
Another similar experiment—			
300 c.mm. ...	52 ,, ,,	52 ,, ,,	52 ,, ,, (72 hours' incubation)

Hence in the rabbit in phosphorus poisoning where the antitrypsin is almost destroyed there is no evidence of activity of an autolytic proteolytic ferment in the serum.

(III) AUTOLYSIS OF SOLID TISSUES, &c.

This investigation was carried out because we desired (1) to determine whether there was any difference in the rate of autolytic degradation of the solid tissues of animals dying from bacterial toxæmia and those of normal animals. The possibility that such differences might occur was suggested by our experiments in which we noted a great increase in the non-coagulable nitrogen in the blood of infected animals, the increase being however almost wholly in the form of urea. The experiments of Jacoby [28] show increased autolytic degradation in phosphorus poisoning; (2) to determine whether serum antitrypsin had any influence on tissue autolysis. The experiments under this head therefore resolved themselves into (*a*) the confirmation or otherwise of the current conception of the rate of autolytic degradation of tissues under conditions of absolute bacteriological sterility; (*b*) to determine whether "antitrypsin" is present in the tissues and if serum antitrypsin has any influence on tissue autolysis.

The great difficulty in these experiments was to obtain the tissues absolutely bacteriologically sterile. In spite of absolute bacteriological sterility in removal and manipulation, it was found that bacteria were present in practically every specimen used. It appears that the liver, especially under normal conditions, contains bacteria, probably in the bile passages only, of the nature of *Bacillus coli* and *Bacillus Welchi*. These bacteria, as will be seen later, have a marked influence on autolytic degradation and account for the current idea of the rapidity of autolytic degradation of the tissues. In order to obtain material bacteriologically sterile, the tissues used were removed as carefully as possible *re* asepsis and were then ground up with sterile normal saline in sterile silica tubes with pebbles, by continued shaking for six hours in a machine shaker—on the principle of the pulverization of bacteria to obtain the "endotoxin." In this way perfectly sterile specimens were obtained for experimentation. N.C.N. was determined by the method previously mentioned.

(A) *Autolysis of the Liver.*

Experiment 1.—An emulsion of normal rabbit's liver was made in normal saline. The emulsion was made about 10 per cent. to compare with the experiments of Schryver [29]. It was found to be bacteriologically sterile.

	Non-coagulable nitrogen
Not incubated ...	8·4 mgr. per 100 c.c. of emulsion
Incubated 4 hours	8·4 ,, ,, ,,
,, 6 ,,	8·4 ,, ,, ,,
,, 8 ,,	8·4 ,, ,, ,,
,, 12 ,,	8·4 ,, ,, ,,
,, 24 ,,	9·6 ,, ,, ,,

Other experiments gave similar results. Rise in twenty-four hours, 7 to 14 per cent. N.C.N.

Experiment 2.—An emulsion of normal rabbit liver was made in normal saline, about 20 grm. in 100 c.c. The liver emulsion proved to be sterile bacteriologically—aërobic and anaërobic tests.

	Non-coagulable nitrogen
(*a*) Control not incubated	17·4 mgr. per 100 c.c. of emulsion
(*b*) ,, incubated 24 hours	18·6 ,, ,, ,,
(*c*) ,, ,, 48 ,,	20·4 ,, ,, ,,

Increase in 24 hours = 7 per cent.
,, 48 ,, = 17 per cent.

Experiment 3.—Another emulsion same strength as Experiment 2.

	Non-coagulable nitrogen
(*a*) Control not incubated	24 mgr. per 100 c.c.
(*b*) ,, incubated 72 hours	29 ,, ,, ,,

= 20 per cent. increase
Acidity at end of 72 hours = n/100 lactic acid

Experiment 4.—A 20 per cent. emulsion of rabbit's liver. Sterile by tests.

	Non-coagulable nitrogen
Control	24 mgr. per 100 c.c.
Incubated 24 hours	27 ,, ,, ,,
,, 72 ,,	31 ,, ,, ,,

Increase in 24 hours = 12·5 per cent.
,, 72 ,, = 28·0 per cent.

Experiment 5.—An experiment—one success amongst many failures—where whole pieces of liver were incubated and proved to be bacteriologically sterile. After incubation the pieces, with exuding fluid, &c., were weighed and ground up in normal saline to make emulsions of equal strength (10 per cent.).

	Non-coagulable nitrogen
Before incubation	68 mgr. per 100 gms. of liver
After 30 hours at 37° C.	84 ,, ,, ,,
,, 4 days ,,	132 ,, ,, ,,
,, 6 ,, ,,	144 ,, ,, ,,

Increase in 30 hours = 24 per cent. of non-coagulable nitrogen = n/50 acidity.
,, 96 ,, = nearly 100 per cent. ,, ,, = n/40 ,,
,, 144 ,, = 110 per cent. of ,, ,, = n/40 ,,

Experiment 6.—An emulsion of liver was made in the usual way with very little saline. It proved to be quite sterile. It was incubated at 37° C. for fourteen days.

Non-coagulable nitrogen
Control, not incubated = 64 mgr. per cent. of the emulsion.
Incubated 14 days = 132 ,, ,, ,,
= over 100 per cent. increase in fourteen days.
Acidity = n/40.

(*B*) *Effect of Added Lactic Acid.*

Experiment 7.—A 20 per cent. emulsion of rabbit's liver was made in normal saline in the usual way. It proved to be bacteriologically sterile. Sterile N/1 lactic acid was added to make the following concentrations. All tests were made up to same bulk with normal saline. Incubation two hours at 37° C.

(1) Control	Not incubated	24 mgr. non-coagulable nitrogen per cent. of emulsion.	
	incubated	24 mgr.	= 0 per cent. rise.
(2) Made n/100 lactic acid		30 ,,	= 25 ,, ,,
(3) Made n/50 ,, ,,		33 ,,	= 37 ,, ,,
(4) Made n/40 ,, ,,		36 ,,	= 50 ,, ,,
(5) Made n/20 ,, ,,		42 ,,	= 75 ,, ,,
(6) Made n/10 ,, ,,		42 ,,	= 75 ,, ,,
(7) Made n/20 lactic acid left at room temperature for twenty minutes, then neutralized and incubated		24 ,,	= 0 ,, ,,

Other experiments gave similar results.

From this experiment it will be seen (1) that the autolytic degradation is proportionate to the degree of acidity and (2) that there is no tissue antitrypsin of the nature of serum antitrypsin since every addition of acid causes increased degradation. There is no degree of acidity below which degradation does not occur and above which it does, as would be the case if a serum antitrypsin were present. Further, the n/20 acid experiment, Experiment 7, No. 7, shows that the autolytic degradation is not due to the destruction of antitrypsin since the degradation has been inhibited by neutralization. Our previous experiments have shown that antitrypsin destroyed by acid cannot be restored by neutralization, so that if the tissue autolysis were solely inhibited by an antitrypsin of this nature, degradation should have taken place, as any such antitrypsin would have been completely destroyed by this treatment. These experiments show further that under absolutely sterile conditions the autolytic degradation of the liver is slow, and this is due to the relatively low concentration of lactic acid in the liver under sterile conditions.

(*c*) The following experiments show how bacterial infection hurries on the process of autolytic degradation, chiefly as the result of the acidity produced by the bacteria from the sugar of the liver and to a less extent due to the proteolytic activity of the infecting organisms.

Experiment 8.—A sterile emulsion of liver 10 per cent. was treated as follows and incubated for four hours :—

	Sterile	n/10 lactic acid	Infected *Bacillus coli* (broth culture ½ c.c. to 10 c.c. of emulsion)
Control	5·76	4·8	6·72
Incubated	5·76	22·1	9·6

milligrammes non-coagulable nitrogen of emulsion.

Lactic acid causes 500 per cent. rise in four hours.

Bacillus coli ,, 50 ,, ,, ,,

Experiment 9.—Similar to above. Four hours' incubation; 10 c.c. of the emulsion in each experiment.

	Sterile and 1 c.c saline	Staphylococci (1 c.c. active broth culture)	Streptococci (1 c.c. active broth culture)	*Bacillus Welchi* (1 c.c. active broth culture)	*Bacillus coli* (1 c.c. active broth culture)
Control	5·6	5·8	5·8	5·8	5·8
Incubated four hours	5·6	5·8	5·8	11·4	9·2

milligrams non-coagulable nitrogen per cent.

Bacillus Welchi alone gives an increase of 68 per cent. N.C.N. in four hours. The broth used was markedly acid, the reaction of the mixture 10 c.c. of emulsion and 1 c.c. of the *Bacillus Welchi* broth was n/120 at the commencement of the experiment.

(*a*) Phosphorus was primarily used during the course of these experiments in the endeavour to obtain bacteriologically sterile livers, the livers from normal animals, as explained above, practically always containing bacteria. The idea was that the excretion of the drug into the bile passages would exercise a bactericidal effect there. We noted that the livers from phosphorus-poisoned rabbits were more frequently sterile than normal. In addition it was used for the purpose of these and other experiments *re* autolysis, &c. In these experiments the rabbits were given 1 c.c. of the oleum phosphoratum B.P. The drug apparently caused the animal no pain, it ate well, &c., till shortly before the fatal issue. The animals were killed at different periods, bled, and the organs removed sterilely.

Experiment 9.—A 20 per cent. emulsion of the liver of a rabbit poisoned with phosphorus was made in sterile normal saline.

Control	24	mgr.	non-coagulable	nitrogen	per cent.	of emulsion.
One hour's incubation at 37° C.	24	,,	,,	,,	,,	,,
Three hours ,, ,,	24	,,	,,	,,	,,	,,
Four ,, ,, ,,	24	,,	,,	,,	,,	,,
Six ,, ,, ,,	24	,,	,,	,,	,,	,,
Eight ,, ,, ,,	24	,,	,,	,,	,,	,,
Twelve ,, ,, ,,	24	,,	,,	,,	,,	,,

Experiment 10.—Similar to Experiment 9, only continued longer. Animal very ill at time of removal of liver.

Control... ...	34	mgr.	non-coagulable	nitrogen	per cent.	of emulsion.
Twenty-four hours	39	,,	,,	,,	,,	,,
Forty-eight hours	49	,,	,,	,,	,,	,,

Increase in twenty-four hours about 15 per cent.
,, ,, forty-eight ,, ,, 44 ,,

Experiment 11.—Solid pieces of liver from a phosphorus poisoned rabbit were removed sterilely, and remained sterile throughout the experiment. At the end of period of incubation the pieces, &c., were weighed and ground up to 20 per cent. emulsion in normal saline. The organ was very fatty. (Animal killed forty-eight hours after inoculation of oleum phosphoratum.)

Control...	42	mgr.	non-coagulable	nitrogen	of emulsion.
Seven hours' incubation at 37° C. ...	42	,,	,,	,,	,,
Twenty-four hours ,, ,, ...	46	,,	,,	,,	,,
				= 10 per cent. increase.	
Seventy-two ,, ,, ,, ..	52	,,	,,	,,	,,
				= 24 per cent. increase.	

Experiment 12.—A liver from a phosphorus-poisoned rabbit was removed sterilely shortly before fatal termination. Pieces were placed in sterile tubes and incubated, then treated as usual.

Control ... ... 108 mgr. non-coagulable nitrogen per cent.
After ninety-six hours ... 108 ,, ,, ,, ,,
Ratio of coagulable to non-coagulable nitrogen before incubation, 0·032.
,, ,, ,, ,, after ,, 0·032.

Here there was no autolysis. Bacteriological sterility during the whole of the experiment. Another specimen of liver gave a similar result.

(*C*) *Liver of Rabbits with Bacterial Toxæmia.*

Experiment 14.—A rabbit was inoculated intravenously with two slopes of dead *Bacillus coli;* after sixteen hours it became very ill, was killed, and the liver removed sterilely. A control rabbit had no food for the same time, to make the experiment strictly comparable. The liver was ground up in the usual way and a 10 per cent. emulsion made with normal saline. During the process of grinding up the tube was placed in an ice jacket. The control liver was treated in the same way.

	Bacillus coli liver Percentage emulsion	Control liver (no food sixteen hours) Percentage emulsion
Control	5·76 mgr. ...	6·78 mgr. non-coagulable nitrogen
One hour	5·76 ,, ...	6·78 ,, ,, ,,
Two hours ...	5·76 ,, ...	6·78 ,, ,, ,,
Three ,, ...	5·76 ,, ...	6·78 ,, ,, ,,
Four ,, ...	5·76 ,, ...	6·78 ,, ,, ,,
Five ,, ...	5·76 ,, ...	6·78 ,, ,, ,,
Six ,, ...	5·76 ,, ...	6·78 ,, ,, ,,
Eight ,, ...	5·76 ,, ...	6·78 ,, ,, ,,
Ten ,, ...	5·76 ,, ...	6·78 ,, ,, ,,

Another experiment gave a like result.

Experiment 15.—Similar to Experiment 14.

	Infected rabbit	Control rabbit
Control	6·8 ...	6·2 mgr. non-coagulable nitrogen per cent.
One hour at 37° C. ...	6·8 ...	6·2 ,, ,, ,, ,,
Two hours ,, ...	6·8 ...	6·2 ,, ,, ,, ,,
Three ,, ,, ...	6·8 ...	6·2 ,, ,, ,, ,,
Four ,, ,, ...	6·8 ...	6·2 ,, ,, ,, ,,
Six ,, ,, ...	6·8 ...	6·2 ,, ,, ,, ,,
Seven and a half hours at 37° C.	7·2 ...	6·2 ,, ,, ,, ,,
Ten hours at 37° C. ...	7·9	6·2 ,, ,, ,, ,,
Twelve ,, ,, ...	8·9 ...	6·2 ,, ,, ,, ,,

But here a growth of *Bacillus coli* was obtained.

Hence it would appear that in phosphorus poisoning and bacterial toxæmia the liver of the infected animals does not autolyse more rapidly *in vitro* than do normal ones, and this in spite of the fact that the blood of infected animals has a great increase in N.C.N. chiefly or almost wholly in the form of urea, which might indicate increased rate of degradation *in vivo*. In fact some of the phosphorus livers do not autolyse at all—it would appear as if the phosphorus had also destroyed the tissue enzymes.

(*D*) *Leucocytes and Marrow.*

Opie [30] in his paper showed that leucocytes easily autolyse in the presence of 0·2 per cent. sodium bicarbonate, less easily in 0·2 per cent. acetic acid. He also showed that the serum antitrypsin exerted an inhibiting influence on the autolysis. He obtained similar results for marrow.

Experiment 16.—We were able to obtain some pus from a case of tubercular psoas abscess. It was quite sterile apart from tubercle bacilli, which were very few. The pus cells were alive, were still capable of active phagocytosis, and were not stained by trypan blue. The cells were broken up into an emulsion (20 per cent.) by shaking as above. Previously they had been centrifuged, the serum removed, and cells washed with sterile normal saline several times.

	Non-coagulable nitrogen
Control	18 mgr. per cent.
Incubated alone seven days at 37° C.	32 ,, ,,
Made n/20 acid	20·4 ,, ,,
Made n/20 alkali	20·4 ,, ,,

We got a similar result in several other experiments made with the same pus.

Experiment 17.—Leucocytes from the peritoneal cavities of several guinea-pigs by aleuron and casein injection. Perfectly sterile. Treated as above.

	Non-coagulable nitrogen
Control	0 mgr. per cent. of emulsion.
Control, incubated at 37° C. for three days ...	6 ,, ,, ,,
,, made 0·2 per cent. lactic acid ...	9 ,, ,, ,,
,, made alkaline 0·2 per cent. sodium carbonate	9 ,, ,, ,,

From which it will be seen that the sterile autolysis of leucocytes is slow, even if acidified or made alkaline.

Experiment 18.—Rabbit marrow was carefully and sterilely removed from the long bones. Emulsified in saline as above. Sterile bacteriologically after emulsification.

		Non-coagulable nitrogen
5 c.c. emulsion—5 c.c. saline,	control	*Nil*
,, ,, ,,	incubated at 37° C. for four days ...	9·6
,, ,, ,,	made 0·2 per cent. Na_2CO_3 for four days...	9·6
,, ,, ,,	made 0·2 per cent. lactic acid	9·6

Similar experiments gave very like results. The marrow of course contained a fair number of red cells.

These results are so at variance with those of other observers that we wondered if there was not some error in our methods. In confirmation, however, of our own results the following experiment can be quoted.

Experiment 19.—A rabbit was killed, bled as much as possible. It was then placed in a strong lysol bath to kill bacteria in the skin; the skin was then drawn off in a room, the air of which was saturated with moisture, and a thigh was amputated sterilely from just below the hip to below the knee; the thigh was then placed in a wide-mouthed glass-stoppered sterile bottle, and the bottle was sealed with paraffin; the thigh was kept in this bottle for eight months: four months at 37° C. in the incubator and four months at summer room temperature (1916). Owing to the above results we examined the thigh at the end of these eight months. The muscle appeared to be perfectly preserved, except that it was a little discoloured and had a steamed appearance There was very little exuded fluid in the jar. On section and microscopic examination the muscle bundles are all perfect, the transverse striation is generally quite perfect, only the nuclei appear diminished in number and shrunken. The interstitial tissue is a little soft, but nerves, blood-vessels, &c., are perfectly visible and appear undegenerated. There was no smell of decomposition; no fatty acid smell. The bone was then opened, and the marrow was seen to be quite firm, only pale. Sections were then made: the drawings herewith are from them. The marrow is almost like the section of fresh marrow—the main change is the absence of red cells; they have all autolysed and are represented by masses of pigment. The marrow cells, &c., have stained perfectly. This shows that absolutely sterile autolysis is extremely slow. This can further be confirmed by post-mortem observation—sterile infarcts of the spleen or kidney do not undergo autolysis at any great rate, there is nothing like a 50 per cent. degradation of the dead tissue in twenty-

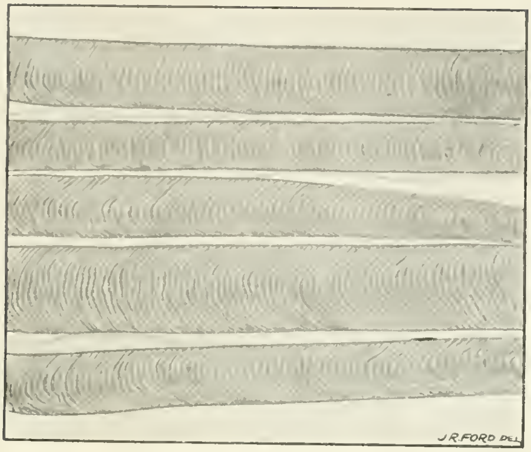


FIG. 1.

Experiment 19. Section of rabbit's muscle incubated for four months at 37° C. and four months at room temperature.

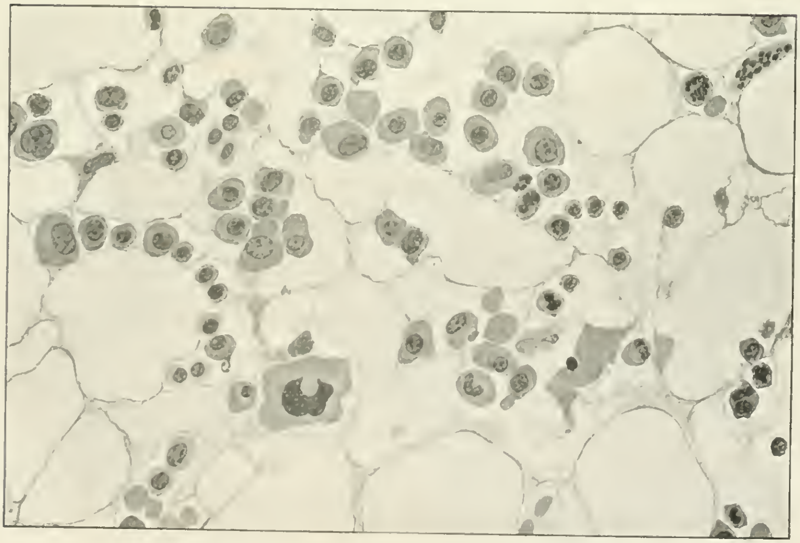


FIG. 2.

Experiment 19. Section of rabbit's marrow incubated at 37° C. for four months and room temperature (summer, 1916) for four months.

four hours (Schryver). Sterile infarcts in fact are only very slowly absorbed. Hence we must conclude from all these experiments that sterile autolysis is very slow, but that autolysis can be greatly accelerated by the presence of bacteria, which act chiefly by the formation of a suitable degree of acidity for the activation of the ferment, and partly in themselves may be capable of digesting the protein.

(IV) Effect of Serum Antitrypsin on Tissue Autolysis.

In the previous section of this paper it has been shown that serum antitrypsin is only definitely destroyed with an acid concentration equal to n/20. If now the autolytic ferment of the tissues is inhibited by an antitrypsin similar in nature to serum antitrypsin it would have been expected that a similar degree of acidity would be needed to destroy the inhibiting antitrypsin. Experiment 7, Section III, however, shows that the rate of autolysis of liver tissue varies with the acid concentration present, and that there is no apparent degree of acid concentration below which autolysis does not occur and above which it occurs, as might be expected if tissue autolysis were inhibited normally by the presence of an antitrypsin of the nature of serum antitrypsin. It has also been shown that antitrypsin once inactivated by acid cannot be reactivated by neutralization; now Experiment 7, Section III, shows that even when a possible antitrypsin has been destroyed by a concentration of n/20 acid, autolysis of the tissue cannot take place when neutralization has been carried out: the antitrypsin if present would have been destroyed, if it were of the nature of serum antitrypsin. The experimental results lead to the conclusion that there is no tissue antitrypsin of the nature of serum antitrypsin, but that the autolytic ferment is normally inhibited by the normal reaction of the tissues.

Experiment 1.—An emulsion of rabbit's liver was made in the usual way and was found to be bacteriologically sterile. Sterile rabbit's serum was used as antitrypsin. A mixture of the two was made in the proportion of 20 c.c. of liver emulsion and 5 c.c. of serum.

	Non-coagulable nitrogen in milligrammes per 100 c.c.
(*a*) Mixture, not incubated	36
(*b*) ,, incubated	36
(*c*) ,, made n/60 lactic acid	36
(*d*) ,, made n/40 ,, ,,	36
(*e*) ,, made n/20 ,, ,,	48

Incubated four hours at 37° C.

Experiment 2.—An emulsion similar to Experiment 1 was used; same proportions of emulsion and serum or saline used.

	Non-coagulable percentage of emulsion
(*a*) Liver emulsion alone + 5 c.c. saline not incubated, made n/40 lactic acid	36
(*b*) Liver emulsion, incubated	54
(*c*) Liver emulsion + 5 c.c. serum, no acid, not incubated ...	36
(*d*) ,, ,, ,, ,, ,, incubated	36
(*e*) ,, ,, made n/40 lactic acid, incubated	36
(*f*) ,, ,, but the serum used was first deantitrypsinized by making n/20 with lactic acid, incubating for one hour at 37° C., then neutralized; the whole afterwards made n/40 lactic acid, incubated ...	36

Incubation four hours at 37° C.

Experiment 3.—Another experiment on similar lines to Experiment 2. In each test was 20 c.c. of a thick liver emulsion, then saline, serum, &c., added and all made n/20 lactic acid by calculation.

	+5 c.c. saline	+ 5 c.c. serum	+ 5 c.c. deantitrypsinized serum	+ 5 c.c. (10 per cent. egg white, fresh)
Control	36	42	42	36
Incubation twenty hours	102	54	54	54
,, seventy-two ,,	138	78	78	72

Non-coagulable nitrogen milligrammes per cent. of the emulsion.

(1) Experiment 1 appears to show an inhibiting influence of the antitrypsin on the autolytic ferment, since with n/20 there is autolysis and below that n/40, &c., which does not destroy antitrypsin, there is none.

(2) Experiment 2 shows that in spite of previously destroying the antitrypsin (*f*) there is no greater degree of autolysis than when the antitrypsin is unaffected (*e*), whereas with n/40 lactic acid alone the liver undergoes marked autolysis.

(3) Experiment 3 shows that when serum is added there is much less autolysis when the reaction is adjusted to n/20 by calculation, and hence antitrypsin inhibited, than when no serum is added. The same is noted even when the antitrypsin of the serum has been previously destroyed. It therefore appears that the restraining action of serum on tissue autolysis is not due to the inhibiting action of serum antitrypsin but to the reaction of the serum affecting the H ion concentration and the consequent differences in this explaining the various results. Further, in the work of previous observers carried out under conditions which were not absolutely bacteriologically sterile the germicidal action of serum greatly helped the idea of an inhibitory activity. We accordingly carried out experiments where the amount of hydrogen ion

was the same in all the experiments. A series of preliminary tests were made. An emulsion of liver was made in normal saline and

(*a*) 4 c.c. and 1 c.c. of saline were made calculated n/20 lactic acid.
(*b*) 4 ,, ,, 1 ,, ,, ,, ,, n/40 ,, ,,
(*c*) 4 ,, ,, 1 ,, of serum ,, ,, n/40 ,, ,,
(*d*) 4 ,, ,, 1 ,, ,, ,, ,, n/40 ,, ,,

To each was added the same amount of a 0·5 per cent. solution of neutral red and a titration made with n/40 NaOH. The amounts required to just make the tubes alkaline to neutral red[1] to the same degree were determined, the differences obtained indicated the amounts of acid bound by the serum and its salts. This amount was added to the amounts previously calculated in each case and new mixtures were thus adjusted till each pair, saline and serum, required the same amount of alkali to produce the same tint in the neutral red in each series. Thus—

Experiment 4.—Rabbit liver emulsion in the proportion of 20 c.c. of emulsion with 5 c.c. of saline or rabbit serum. Sterile experiment.

	(*a*)	(*b*)	(*c*)	(*d*)
	Saline added calculated n/20	Serum added reaction = to (*a*)	Saline added calculated n/40	Serum added reaction = to (*c*)
Not incubated ...	30 ...	36 ...	30 ...	36
Incubated six hours ...	60 ...	69 ...	48 ...	55
,, twenty-four hours	108 ...	120 ...	84 ...	96
	milligrams non-coagulable nitrogen per cent. of emulsion.			
	Per cent.	Per cent.	Per cent.	Per cent.
Increase in six hours ...	100 ...	91 ...	60 ...	53
,, twenty-four hours	260 ...	250 ...	180 ...	166
	as just determined, using neutral red as indicator.			

Experiment 5.—An emulsion of freshly killed chicken liver was made and found to be sterile. It was mixed as follows in proportion of 20 c.c. of the emulsion to 5 c.c. of the other constituents.

	Non-coagulable nitrogen milligrams per cent.		
	Control	Incubation	Percentage rise
(*a*) Liver + saline, made nominal n/20 lactic acid ...	25·2 ...	60·0 ...	= 140·0
(*b*) Liver + 5 c.c. egg antitrypsin titre, made nominal n/20 ...	27·6 ...	50·4 ...	= 80·0
(*c*) Liver + chicken's serum, made nominal n/20 ...	27·6 ...	42·0 ...	= 50·0
(*d*) Liver + chicken's serum, made equal in free acid to the liver and saline tube (*a*) ...	27·6 ...	67·2 ...	= 140·0
(*e*) Liver + antitrypsin, made equal in free acid to the liver and saline (*a*) ...	27·6 ...	64·0 ...	= 130·0

---

[1] This was used since it was the only indicator available.

Hence here the egg antitrypsin does not inhibit autolysis of chicken liver. Again the result depends on the degree of ionization.

Thus serum antitrypsin exercises no restraining influence on the autolysis of tissues such as the liver. From Opie's [30] experiments, however, it appears that the serum antitrypsin inhibits the leucocyte ferments since he shows that if an effusion containing leucocytes is incubated no autolysis occurs, but if the effusion is first centrifuged, the serum removed and the leucocytes washed, autolysis occurs. In the following experiments the influence of serum antitrypsin on leucocyte ferment is shown.

Experiment 6.—Sterile pus from a psoas abscess. The pus was centrifuged, the supernatant serum removed and put aside, the leucocytes repeatedly washed in sterile normal saline. A 20 per cent. emulsion was made in normal saline: 15 c.c. were used in each test. Mixtures incubated for twenty-four hours.

			Milligrams non-coagulable nitrogen per cent.
(*a*)	Leucocytes	+ 1 c.c. of normal saline (Control)	= 16
(*b*)	,,	+ 1 c.c. of normal saline, incubated	= 22
(*c*)	,,	+ 1 c.c. of supernatant fluid, incubated	= 18
(*d*)	,,	+ 1 c.c. of supernatant fluid, heated to 60° C. for thirty minutes, incubated	= 20
(*e*)	,,	+ 1 c.c. of supernatant fluid (Control)	= 17
(*f*)	,,	+ 1 c.c. of human serum, incubated	= 17
(*g*)	,,	+ 1 c.c. of human serum, heated to 60° C. for thirty minutes, incubated	= 21
(*h*)	,,	+ 1 c.c. of human serum (Control)	= 17

Control = not incubated.

Conclusions.

This paper deals with the nature of serum antitrypsin showing:—

(1) That it is protein in nature and not lipoidal.

(2) That it is not adsorbed by bacteria or colloids.

(3) That there is a weak proteolytic ferment present in the serum.

(4) That it is destroyed by 56° C. and by weak acids and alkalis to N/400.

(5) That it does not influence tissue autolysis.

Also the paper deals with tissue autolysis, and shows that this is quite slow under conditions of absolute bacteriological sterility. Further the paper deals with the question of autolytic degradation occurring in infection and anaphylaxis and is opposed to the work of Jobling bearing on these points.

REFERENCES.

[1] Teale and Embleton. *Lancet*, 1913, i, p. 234.
[2] Vaughan. *Journ. Amer. Med. Assoc.*, 1914, lxii, p. 583.
[3] Jobling and Petersen. *Journ. Exper. Med.*, 1914, xix, pp. 459 *et seq.*
[4] Wright. *Lancet*, 1917, i, p. 6.
[5] Hildebrandt. *Virchow's Archiv*, 1893, cxxxi, p. 5.
[6] Hahn. *Berl. klin. Wochenschr.*, 1917, p. 499.
[7] Levine and Sankey. *Journ. Med. Research*, 1903, x, p. 127.
[8] Weinland. *Zeit. f. Biol.*, 1903, xliv, p. 45.
[9] Hamill. *Journ. Physiol.*, 1906, xxxiii, p. 429.
[10] Cathcart. *Journ. Physiol.*, 1905, xxxi, p. 491.
[11] Achalme. *Ann. de l'Inst. Pasteur*, 1901, xv, p. 737.
[12] Jochmann and Kantorowicz. *Münch. med. Wochenschr.*, 1908, lv, p. 728.
[13] Mayer, K. "Folia Serologica," 1911, ii, p. 472.
[14] Eisner, G. *Zeit. f. Immunitätsf. O.*, 1908-9, i, p. 650.
[15] Wiens. *Münch. med. Wochenschr.*, 1907, liv, p. 2637.
[16] Delezenne and Pozerski. *Comptes rend. Soc. Biol.*, 1903, lv, p. 327.
[17] Bayliss and Starling. *Journ. Physiol.*, 1905, xxxii, p. 129.
[18] Rosenthal. "Folia Serologica," 1910, vi, p. 285.
[19] Bayliss. *Archives de Soc. Biol.*, 1904, xi (suppl.), p. 261.
[20] Abderhalden and Gigon. *Zeit. f. physiol. Chemie*, 1907, liii, p. 251.
[21] Walters. *Journ. Biol. Chem.*, 1912, xii, p. 43.
[22] Schwarz. *Wiener. klin. Wochenschr.*, 1909, xxii, p. 1151.
[23] Sugimoto. *Arch. f. Exp. Path. u. Pharm.*, 1913, lxxiv, p. 14.
[24] Jobling. *Journ. Exper. Med.*, 1914, xix, pp. 239 and 459.
[25] Cobliner, S. *Biochem. Zeitschr.*, 1910, xxv, p. 494.
[26] Cathcart. *Journ. Physiol.*, 1904, xxxi, p. 497.
[27] Abderhalden. Abwehrfermente, 1913.
[28] Jacoby. *Zeit. f. physiol. Chemie*, 1900, xxx, p. 149.
[29] Schryver. *Journ. Physiol.*, 1905, xxxii, p. 159.
[30] Opie. *Journ. Exper. Med.*, 1905, vii, p. 316 and 795.

John Bale, Sons & Danielsson, Ltd., 83-91 Great Titchfield Street, London, W.1.

The Relation of the Antitryptic Titre of the Blood to Bacterial Infection and Anaphylaxis.[1]

By F. H. Teale and E. Bach.

From the Research Laboratories, University College Hospital Medical School, London.[2]

In this paper we propose recording our observations on the variations in the antitryptic titre of the blood of animals during various stages and degrees of bacterial infection and anaphylactic shock. In addition we include observations on the alteration in the antitryptic titre of the serum when anaphylotoxin is produced *in vitro*. So far we have been unable to find any records of systematic observations regarding variations in the antitryptic titre of the blood in infection, &c., the observations are chiefly scattered clinical observations, there are no records of continued observations.

In these experiments we have investigated the changes in the antitryptic power of the blood in—

(I) Normal animals infected with or inoculated with—
    (A) Non-pathogenic bacteria.
    (B) Heterogeneous non-toxic protein.
    (C) Pathogenic and virulent bacteria.
    (D) Exotoxins.
    (E) Sensitized bacteria.

(II) Immunized animals reinjected with the homologous bacteria.

(III) Sensitized or immunized animals reinjected with the homologous antigen.

(IV) Variations due to (*a*) food; (*b*) phosphorus poisoning, &c.; (*c*) agents destroying antitrypsin *in vitro*; (*d*) drugs affecting the temperature; (*e*) drugs damaging blood-forming tissue.

(V) Variation in the *in vitro* preparation of anaphylotoxin.

(VI) Influence of antitrypsin on the growth of bacteria.

---

[1] At a meeting of the Section, held December 2, 1919.

[2] The expenses of this research were defrayed by a grant from the Medical Research Committee.

Method.

It has been shown in a previous paper (1) that antitrypsin is not affected by keeping, especially at 0° to 3° C. In order to reduce errors due to strength of trypsin, &c., the samples of serum obtained from a given animal were all stored in this way till the series was complete, and all were tested together, using for the experiment the same gelatine, trypsin, &c.

The method adopted was as stated previously (2). The unit of trypsin was determined as that amount of a 1 per cent. solution which caused complete liquefaction of 1 c.c. of 15 per cent. gelatine in thirty minutes at 37° C. in the water bath. The tubes after incubation were kept at 10° C. for thirty minutes before the reading was taken. The unit of trypsin thus determined was placed in a series of small tubes and graded quantities of the serum to be tested added (the serum was diluted 1 : 2 or 1 : 5 according to its titre; which in some cases was determined roughly first). These mixtures were incubated for thirty minutes at 37° C. in the water bath, then 1 c.c. of 15 per cent. gelatine at 37° C. added, and a further period of thirty minutes' incubation in the water bath at 37° C. allowed—then cooled at 10° C. for thirty minutes. The titre was the smallest amount of serum completely inhibiting liquefaction by the trypsin.

(1) Normal Animals infected with or inoculated with—

(A) Non-pathogenic Bacteria.

Experiment 1: Sarcina lutea.—A rabbit of 1,920 grm. inoculated intravenously with one slope of *Sarcina lutea*.

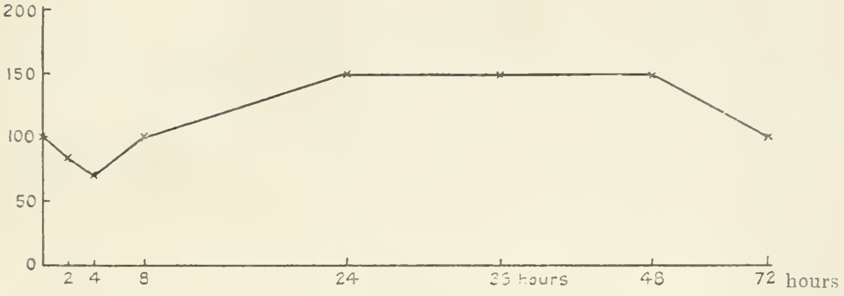
Antitryptic titres		Before inoculation	3	6	12	24	38	48	60 hours after inoculation
Unit in	...	80	100	100	80	60	55	55	80 c.mm. of serum

Experiment 2: Sarcina lutea.—A quarter slope of a twenty-four hours' laboratory strain was inoculated intravenously into a rabbit.

Titre		Before inoculation	3	6	9	24	48	72 hours after inoculation
Unit in		60	60	80	80	40	30	40 c.mm. of serum

Experiment 3: Bacillus mycoides.—Growing at 22° C. Two slopes were inoculated into a rabbit of 1,080 grm.

Antitryptic titre		Before inoculation	2	4	8	24	36	48	72 hours after inoculation
Unit in	...	60	70	80	60	40	40	40	60 c.mm. of serum



Experiment 3. Rabbit inoculated with two slopes of *Bacillus mycoides*, 22° C. intravenously.

Experiment 4: Bacillus smegma.—Two slopes were inoculated intravenously into a rabbit of 1,860 grm.

Titre	Before inoculation	3	6	9	18 hours (death) after inoculation
Unit in ...	60	55	50	45	35 c.mm. of serum

Experiment 5: Bacillus prodigiosus.—Two slopes of *Bacillus prodigiosus* were inoculated intravenously into a rabbit of 1,960 grm.

Antitryptic titres	Before inoculation	3	6	10	24	48	72 hours after inoculation
Unit in ...	60	80	80	60	40	40	50 c.mm. of serum

(B) PRIMARILY NON-TOXIC PROTEINS.

(1) *Sheep's Red Corpuscles, washed Sterile with Sterile Normal Saline.*

Experiment 6.—10 c.c. of sheep's sterile red cells were inoculated intraperitoneally into a rabbit.

Antitryptic titre	Before inoculation	3	6	9	24	36	48	72 hours after inoculation
Unit in ...	60	80	80	60	35	35	30	30 c.mm. of serum.

Experiment 7.—Sterile washed sheep's red cells: 10 c.c. were inoculated intraperitoneally into a rabbit of 2,480 grm.

Antitryptic titre	Before inoculation	3	9	24	48	90 hours after inoculation
Unit in ..	60	60	40	30	30	60 c.mm. of serum

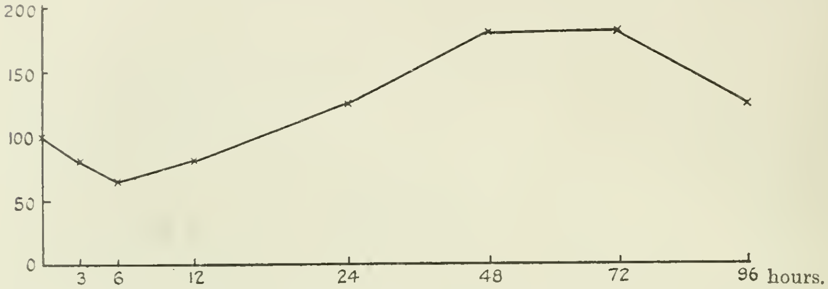
Experiment 8.—5 c.c. of sterile sheep's red cells were inoculated intravenously into a rabbit of 2,020 grm.

Antitryptic titre	Before inoculation	2	4	6	20	36	54	72	96	120 hours after inoculation
Unit in ...	60	60	80	80	40	30	30	30	60	60 c.mm. of serum

(2) *Sterile Egg Albumen.*

Experiment 9.—5 c.c. of a 10 per cent. suspension of sterile egg albumen was inoculated intravenously into a rabbit of 2,860 grm.

Antitryptic titre		Before inoculation	3	6	12	24	48	72	96 hours after inoculation
Unit in	...	50	60	80	60	40	30	30	40 c.mm. of serum



Experiment 9. Rabbit inoculated with 5 c.c. 10 per cent. egg albumen intravenously.

Experiment 10.—Similar experiment to No. 9, only 5 c.c. of a 1 per cent. suspension was injected. Rabbit of 1,800 grm.

Antitryptic titre		Before inoculation	3	6	10	24	36	48	72 hours after inoculation
Unit in	...	60	70	70	60	50	40	40	50 c.mm. of serum

Experiment 11.—Similar experiment to No. 9, only 1 c.c. of 0·1 per cent. solution was used. Rabbit of 1,960 grm.

Antitryptic titre		Before inoculation	3	6	9	20	36	48 hours after inoculation
Unit in	...	60	60	80	80	40	40	60 c.mm. of serum.

(3) *Sterile Gelatine.*

Experiment 12.—Rabbit of 2,000 grm. was inoculated with 20 c.c. of 30 per cent. gelatine intraperitoneally.

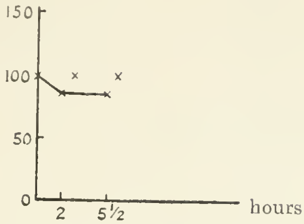
Antitryptic titre		Before inoculation	3	6	12	24	48	72 hours after inoculation
Unit in	...	80	100	100	80	60	40	60 c.mm. of serum

(C) PATHOGENIC BACTERIA.

(1) *Experiments with Bacillus coli.*

Experiment 13.—Four slopes of virulent *Bacillus coli* were inoculated intravenously into a rabbit of 2,500 grm. Death occurred in five and a half hours.

Antitryptic titre of serum	Before inoculation	2 hours after	After death 5½ hours
Unit in ...	70 ...	80 ...	80 c.mm. of serum



Experiment 13. Rabbit inoculated intravenously with four slopes of *Bacillus coli*; death in five and a half hours.

Experiment 14.—Four slopes of virulent *Bacillus coli* were inoculated intravenously into a rabbit of 1,800 grm. Death occurred in two hours. The animal soon showed signs of toxæmia.

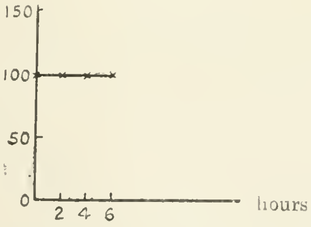
Antitryptic titre	Before inoculation	30 minutes	1 hour	2 hours after inoculation
Unit in ...	60	60	75	75 c.mm. of serum

Experiment 15.—A rabbit of 2,200 grm. had six slopes of non-virulent *Bacillus coli* inoculated intravenously. Death occurred in six hours.

Antitryptic titre	Before inoculation	3 hours after	Immediately after death, 6 hours
Unit in ...	60 ...	60 ...	60 c.mm. of serum

Experiment 16.—A rabbit of 1,800 grm. was inoculated with four slopes of non-virulent *Bacillus coli* intravenously. Death occurred in six hours.

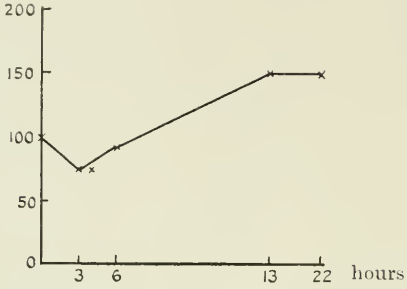
Antitryptic titre	Before inoculation	2	4	6 hours after inoculation
Unit in ...	60	60	60	60 c.mm. of serum



Experiment 16. Rabbit inoculated intravenously with four slopes of *Bacillus coli*; death in six hours.

Experiment 17.—A rabbit of 2,080 grm. was inoculated with four slopes of an ordinary laboratory strain of *Bacillus coli* intravenously. Death occurred in twenty-two hours.

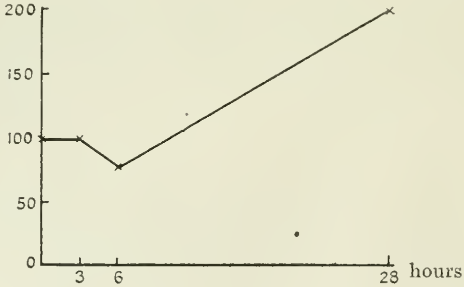
Antitryptic titre	Before inoculation	3	6	18	22 hours after inoculation
Unit in ...	60	80	70	40	40 c.mm. of serum



Experiment 17. Rabbit inoculated with four slopes of *Bacillus coli* intravenously; death in twenty-two hours.

Experiment 18.—A rabbit of 1,720 grm. was inoculated intravenously with four slopes of dead *Bacillus coli*, the same strain as Experiment 15. Recovery.

Antitryptic titre	Before inoculation	3	6	28 hours after inoculation
Unit in ...	80	80	100	40 c.mm. of serum



Experiment 18. Rabbit inoculated with four slopes of dead *Bacillus coli* intravenously; recovery.

Experiment 19.—A rabbit of 2,210 grm. was inoculated with quarter slope of very virulent *Bacillus coli*. Death in thirty-six hours.

Antitryptic titre	Before inoculation	3	6	12	24 hours after inoculation (death)
Unit in	60	60	80	60	40 c.mm. of serum
Bacteria in circulation	—	+	—	—	+

(2) *Experiments with Bacillus typhosus.*

Experiment 20.—A rabbit of 1,980 grm. was inoculated intravenously with two slopes of a twenty-four hours' growth of *Bacillus typhosus* on agar. Recovery.

Antitryptic titre	Before inoculation	2	4	8	10	24 hours after inoculation
Unit in	40	60	60	60	55	55 c.mm. of serum
Bacteria in peripheral blood	·	+	+	−	+	—

Experiment 21.—A rabbit of 2,180 grm. was inoculated with two slopes of *Bacillus typhosus* as in Experiment 20, intravenously. Recovery.

Antitryptic titre	Before inoculation	3	6	10	24	48 hours after inoculation
Unit in	60	80	80	60	40	40 c.mm. of serum
Bacteria in peripheral blood		+	+	−	−	

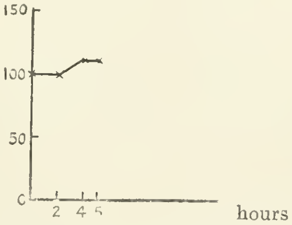
(3) *Experiment swith Varieties of Bacillus mycoides of Varying Virulence* [3].

Experiment 22.—A normal rabbit of 2,018 grm. was inoculated intravenously with two slopes of slightly virulent *Bacillus mycoides* (37° C). Death occurred in sixty-six hours.

Antitryptic titre	Before inoculation	2	4	8	9	24	48	60	66 hours after inoculation
Unit in	60	60	80	55	55	50	40	40	50 c.mm. of serum
Bacteria in peripheral blood		−	+	−	+	−	−	+	+

Experiment 23.—A rabbit of 1,096 grm. was inoculated intravenously with one slope of naturally warm growing *Bacillus mycoides* (37° C.) isolated from a wound. The rabbit died in five hours.

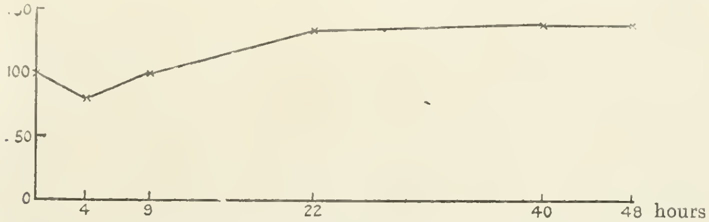
Antitryptic titre	Before inoculation	2	4	5 hours after inoculation
Unit in ...	60	60	55	55 c.mm. of serum



Experiment 23. Rabbit inoculated with one slope of *Bacillus mycoides* (37° C.); death in five hours.

Experiment 24.—A rabbit was inoculated with four slopes of *Bacillus mycoides* educated to grow at 37° C. The animal died in seventy-two hours. Organisms were present in the tissues post-mortem in small numbers.

Antitryptic titre	Before inoculation	4	9	22	48	72 hours after inoculation
Unit in	40	50	40	30	28	28 c.mm. of serum
Bacteria in peripheral blood		−	+	−	−	+ (very few)



Experiment 24. Rabbit inoculated with four slopes of *Bacillus mycoides* (37° C.) intravenously; death in seventy-two hours.

(4) *Experiments with Bacillus anthracis.*

Experiment 25.—A rabbit of 2,080 grm. was inoculated with one slope of *Bacillus anthracis.* Death occurred in 60 hours.

Antitryptic titre	Before inoculation	3	5	10	24	30	48 (death)	hours after inoculation
Unit in	60	80	80	70	50	40	40	40 c.mm. of serum
Bacteria in peripheral blood		+	+ (very few)	+	−	−	+ (very numerous)	

(5) *Virulent Streptococci.*

Experiment 26.—A rabbit was inoculated intravenously with two slopes of virulent streptococci. Death occurred in twenty-two hours.

Antitryptic titre	Before inoculation	3	6	10	22 hours after inoculation
Unit in	60	80	60	40	40 c.mm. of serum
Bacteria in peripheral blood	−	+	+ (very few)	+	+

Experiment 27.—A rabbit was inoculated intravenously with $\frac{1}{4}$ c.c. of a twenty-four hours' broth culture of virulent streptococcus. Animal very ill, but recovered.

Antitryptic titre	Before inoculation	3	10	24	30	48	72 hours after inoculation
Unit in ...	60	60	80	50	40	40	50 c.mm. of serum

(6) *Pneumococci.*

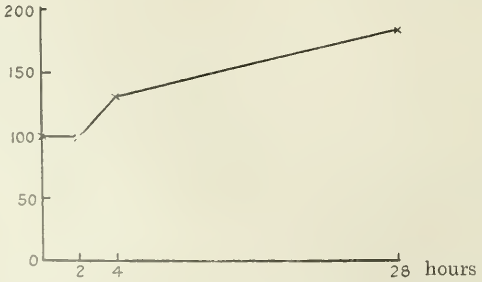
Experiment 28.—A rabbit was inoculated with a virulent broth culture of the pneumococcus. Death occurred in thirty-six hours.

Antitryptic titre	Before inoculation	3	6	9	24	30 hours after inoculation (at death)
Unit in ...	60	80	80	60	40	40 c.mm. of serum

(7) *Bacillus enteritidis Gaertner.*

Experiment 29.—A rabbit was inoculated intravenously with one slope of *Bacillus enteritidis* of Gaertner. Death occurred in twenty-eight hours.

Antitryptic titre	Before inoculation	2	6	28 hours after inoculation
Unit in ...	90	90	70	60 c.mm. of serum



Experiment 29. Rabbit inoculated with one slope of *Bacillus enteritidis Gaertner*; death in twenty-eight hours.

(D) EXOTOXINS.

Experiment 30.—A rabbit of 2,200 grm. was inoculated intravenously with 10 c.c. of tetanus toxin. Death in eighteen hours.

Antitryptic titre		Before inoculation	2	4	7	18 hours after inoculation
Unit in	...	60	60	70	60	40 c.mm. of serum

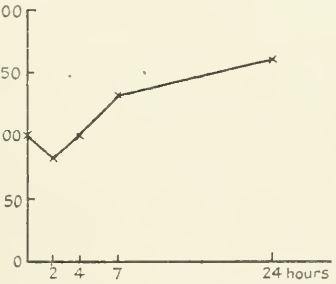
Experiment 31.—A rabbit was inoculated with 7 c.c. of diphtheria toxin, intravenously. Death in twenty-four hours.

Antitryptic titre		Before inoculation	3	6	17	24 hours after inoculation
Unit in	...	80	80	120	80	80 c.mm. of serum

(E) SENSITIZED BACTERIA.

Experiment 32.—A rabbit was inoculated intravenously with two slopes of sensitized typhoid bacilli. Recovery. The typhoid bacilli were exposed to a large quantity of high grade agglutinating serum for twenty-four hours at room temperature.

Antitryptic titre		Before inoculation	2	4	7	24 hours after inoculation
Unit in	...	40	50	40	35	25 c.mm. of serum



Experiment 32. Rabbit inoculated intravenously with two slopes of fully sensitized *Bacillus typhosus.*

Experiment 33.—Experiment as above. A rabbit of 1,920 grm. was inoculated intravenously with two slopes of fully sensitized typhoid bacilli.

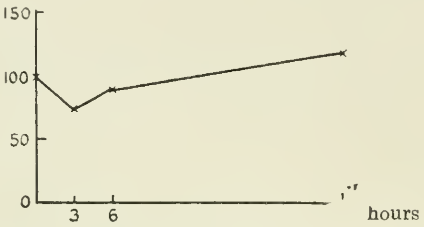
Antitryptic titre		Before inoculation	3	6	10	24	48	72	96	120 hours after inoculation
Unit in	...	60	60	60	50	40	40	40	50	60 c.mm. of serum

Experiment 34 : Sensitized Pneumobacilli.—A rabbit was inoculated intravenously with one slope of sensitized pneumobacilli Recovery. Sensitization as before.

Antitryptic titre		Before inoculation	3	5	10	24	48	72	96 hours after inoculation
Unit in	...	40	50	45	40	30	25	30	40 c.mm. of serum

Experiment 35: Sensitized Bacillus enteritidis Gaertner.—A rabbit was inoculated intravenously with one slope of highly sensitized *Bacillus enteritidis Gaertner.* It died in twenty-four hours.

Antitryptic titre	Before inoculation	3	6	24 hours after inoculation
Unit in ...	60	80	70	50 c.mm. of serum



Experiment 35. Rabbit inoculated intravenously with one slope of fully sensitized *Bacillus enteritidis Gaertner*; death in twenty-four hours.

(II) Inoculation into Immune and Sensitized Animals.

Experiment 36.—A rabbit of 1,840 grm. with an agglutinin titre against *Bacillus typhosus* of 1 : 10,000 was inoculated intravenously with two slopes of *Bacillus typhosus.* Recovery.

Antitryptic titre	Before inoculation	2	4	8	10	25	48	72 hours after inoculation
Unit in	40	45	40	50	45	30	30	40 c.mm. of serum
Bacteria in peripheral circulation	—	+	+	+	—	—	—	—

Experiment 37.—A rabbit of 2,010 grm. was immunized against the pneumobacillus. Later two slopes of pneumobacilli were inoculated intravenously. Recovery.

Antitryptic titre	Before inoculation	3	6	9	24	48	72	96 hours after inoculation
Unit in	40	40	45	40	30	25	25	30 c.mm. of serum
Bacteria in peripheral circulation	—	+	+	—	—	—	—	—

Experiment 38.—A rabbit was immunized against the *Bacillus mycoides*: 200 mgr. dead bacteria were first inoculated intraperitoneally then two slopes of the organism grown at 37° C. ten days after. Finally, three weeks after last injection two slopes of virulent *Bacillus mycoides* were inoculated intravenously. Death in ninety-six hours.

Antitryptic titre	Before inoculation	3	7	24	30	45	60	96 hours after inoculation
Unit in	50	60	60	50	40	30	30	50 c.mm. of serum

Experiment 39.—Another rabbit was immunized with 200 mgr. dead *Bacillus mycoides*, intraperitoneally. Ten days later two slopes of

virulent *Bacillus mycoides* intravenously, and seven days later two slopes of virulent *Bacillus mycoides* intravenously. Death in seventy-two hours after last injection.

Antitryptic titre	Before inoculation	2	5	9	24	48	72 hours after inoculation
Unit in	40	60	50	50	40	30	30 c.mm. of serum
Bacteria in peripheral circulation	−	+	+	+	−	−	+

Experiment 40.—A rabbit had 200 mgr. dead *Bacillus mycoides* inoculated intraperitoneally. Ten days later two slopes of virulent *Bacillus mycoides* intravenously. Death in eight hours.

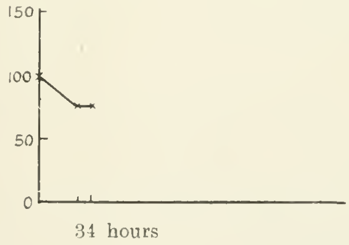
Antitryptic titre	Before inoculation	1	3	5 hours after inoculation
Unit in ...	70	72	72	72 c.mm. of serum

Experiment 41.—A rabbit was inoculated intraperitoneally with 200 mgr. dead *Bacillus mycoides*, followed in ten days by a further 200 mgr. Then ten days later two slopes of non-virulent but 37° C. growing *Bacillus mycoides* were inoculated intravenously. Recovery.

Antitryptic titre	Before inoculation	2	4	6	24	48	72	96 hours after inoculation
Unit in	70	70	70	60	50	45	40	45 c.mm. of serum
Bacteria in circulation	−	+	+	+	−	−	+	−

Experiment 42.—Rabbit immunized against *Bacillus enteritidis Gaertner*. Titre 1 : 50,000. Inoculated intravenously with one slope of *Bacillus enteritidis Gaertner*. Died in four hours.

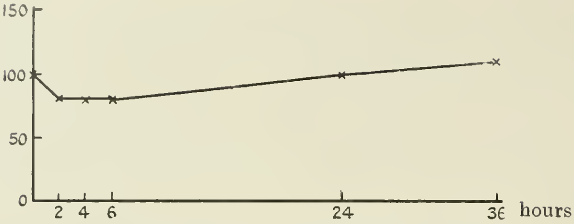
Antitryptic titre	Before inoculation	3	4 hours after inoculation
Unit in	60	80	80 c.mm. of serum



Experiment 42. Immune rabbit inoculated intravenously with one slope of *Bacillus enteritidis Gaertner*; death in four hours.

Experiment 43.—Similar experiment to above. Animal lived thirty-six hours.

Antitryptic titre	Before inoculation	2	4	6	24	30	36 hours after inoculation
Unit in ...	40	50	50	50	40	35	35 c.mm. of serum



Experiment 43. Immune rabbit inoculated intravenously with *Bacillus enteritidis Gaertner;* death in thirty-six hours.

Experiment 44. — One slope of sensitized *Bacillus enteritidis Gaertner* was inoculated intravenously into a rabbit immunized against *Bacillus enteritidis Gaertner.* Died in fifty-two hours.

Antitryptic titre	Before inoculation	3	6	9	24	48	52 hours after inoculation
Unit in ...	80	80	90	100	75	50	50 c.mm. of serum

(III) RABBITS SENSITIZED AGAINST EGG ALBUMEN.

Experiment 45.—Rabbits each received 5 c.c. of 10 per cent. suspension of egg albumen intraperitoneally. Twenty-one days later they each received as follows:—

Rabbit	Antitryptic titre Before injection		Antitryptic titre After death
(1) 0·5 c.c. fresh egg albumen intravenously, death in two minutes	40	...	30 c.mm.
(2) 0·5 c.c. 20 per cent. egg albumen intravenously, death in ten minutes	50	...	40 ,,
(3) 0·5 c.c. 10 per cent. egg albumen; very ill, recovered.			

Antitryptic titre	Before inoculation	½	1	4	7	24 hours after inoculation
Unit in ...	40	45	50	40	30	30 c.mm. of serum

Experiment 46.—A rabbit was inoculated with 10 c.c. of pure egg albumen intraperitoneally. Fourteen days later 0·25 c.c. of pure egg albumen was inoculated intravenously. The animal became very ill, temperature fell to below 95° F., rapid breathing, &c.

Antitryptic titre	Before inoculation	½	1	2	4	8	24	48 hours after inoculation
Unit in ...	50	50	60	70	70	80	55	55 c.mm. of serum

Experiment 47.—A series of guinea-pigs was inoculated each with 0·5 c.c. of pure egg white. Three weeks later they were injected as follows: sufficient blood was obtained before the operation for the control antitryptic titre.

	Antitryptic titre	
	Before inoculation	After inoculation
(1) Control; 0·5 c.c. of 10 per cent. egg albumen intravenously; death in two minutes	—	—
(2) Received 0·5 c.c. of 10 per cent. egg albumen intravenously; death in two minutes	50	50
(3) Received 0·5 c.c. of 10 per cent. egg albumen intravenously; death in ten minutes	50	50
(4) Received 0·25 c.c. of 10 per cent. egg albumen intravenously; killed after thirty minutes	55	85
(5) Received 0·25 c.c. of 10 per cent. egg albumen intravenously; killed one hour after	50	70
(6) Received 0·25 c.c. of 10 per cent. egg albumen intraperitoneally: killed one hour after	40	60
(7) Normal pig; 0·5 c.c. egg albumen intravenously; killed one hour after	50	70

Experiment 48.—Rabbit immunized against sheep's serum. Titre of rabbit's serum 1 : 10,000; 2 c.c. of sheep serum inoculated intravenously.

Titres	Before inoculation	Minutes			Hours after inoculation					
		5	15	30	3	6	12	24	48	
Unit in	80	80	90	100	100	100	80	60	50	c.mm. of serum

Experiment 49.—Rabbit immunized against sheep's red cells. Titre 1 : 3,000; 2 c.c. of sheep's washed red cells inoculated intravenously. Animal had fall of temperature, rapid breathing, &c.

Titres		Before inoculation	3	6	12	24	36	48	72 hours after inoculation
Unit in	...	60	60	60	50	40	40	40	50 c.mm. of serum

Experiment 50.—Another rabbit immunized against sheep's red cells. Titre of serum 1 : 5,000; 5 c.c. of sheep's red cells inoculated intraperitoneally. The animal became very ill, but recovered.

Titre		Before inoculation	3	6	9	24	48	72 hours after inoculation
Unit in	...	60	70	80	60	40	30	40 c.mm. of serum

(IV) INFLUENCE OF FOOD, &C.

(*a*) During infections animals do not take food, hence it was necessary to study the effect of starvation and food on the antitryptic titre.

Experiment 51.—Rabbit, 2,860 grm. had no food, only water in cage for forty-eight hours after a full meal, then food was given. The animal took water freely.

Titre		Before inoculation	12	24	36	48	Food 48	50	Food 58	72	96 hours after inoculation
Unit in	...	60	55	55	55	60	—	80	100	100	70 c.mm. of serum

Experiment 52.—Rabbit, 2,720 grm.; no solid food for sixty-eight hours, only water, food continuously after.

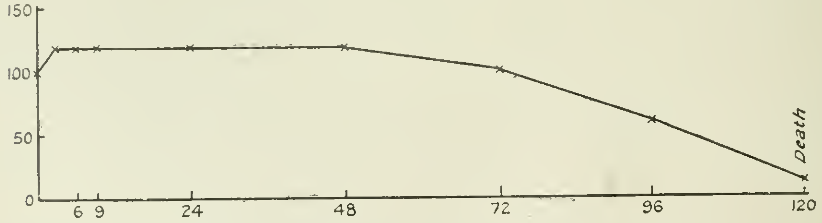
Titre	Before inoculation	24	45	53	Food 68	72	96 hours after inoculation
Unit in ...	60	55	65	65	85	85	75 c.mm. of serum

These experiments appear to show that a period of forty-eight hours' abstinence from food has no marked effect on the antitryptic titre of the blood, and that therefore the abstinence from food during toxæmia is not responsible for the alteration in the antitryptic titre.

(*b*) In phosphorus poisoning there appears to be evidence of autolytic degradation of the tissues, hence it seemed important to ascertain the changes in the antitryptic titre following the injection of oleum phosphoratum B.P.

Experiment 53.—A rabbit was injected subcutaneously with 1 c.c. of oleum phosphoratum B.P. The animal suffered no pain and ate well up to the evening before death.

Antitryptic titre	Before inoculation	3	6	9	24	48	72	96	120 hours after inoculation
Unit in ...	60	50	50	50	50	50	60	100	not in 500 c.mm. of serum



Experiment 53. Rabbit inoculated subcutaneously with 1 c.c. Ol. phosphoratum (B.P.).

Experiment 54.—Similar experiment. Rabbit inoculated subcutaneously with 1 c.c. of oleum phosphoratum B.P. Death in eighty hours.

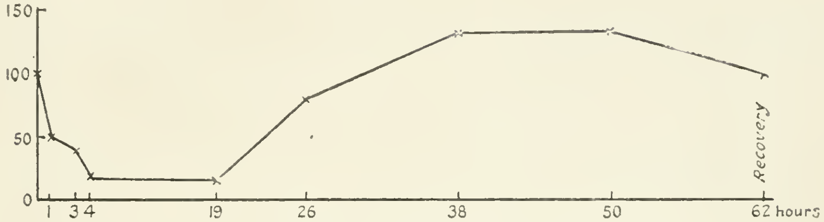
Antitryptic titre	Before inoculation	3	9	24	26	48	72	80 hours after inoculation
Unit in ...	60	60	60	50	50	60	100	300 c.mm. of serum

c) *Effect of Reagents destroying the Antitrypsin in vitro, on the Titre in vivo.*

(1) Lactic acid.

Experiment 55.—A rabbit of 2,000 grm. was inoculated intravenously with 5 c.c. of n/1 lactic acid. This was followed by great rapidity of breathing and air-hunger for ten minutes; the animal then rapidly recovered and there was no subsequent ill effect.

Non-coagulable nitrogen in the blood: Rise in four hours, 57 per cent.; in five hours, 77 per cent.



Experiment 55. Rabbit inoculated intravenously with 5 c.c. N/1 lactic acid.

Titre of blood	Before inoculation	Minutes		Hours after inoculation								
		15	30	1	3	4	19	26	38	50	62	
Unit in ...	80	120	140	200	200	400	400	100	60	60	80	c.mm. of serum

Experiment 56.—A rabbit was kept continuously under the influence of ether for one hour, continually inhaling some.

Antitryptic titre	Before inoculation	1	3	6	24	48 hours after inoculation
Unit in ...	80	80	80	80	60	60 c.mm. of serum

Experiment 57.—A rabbit of 2,180 grm. received a solution containing 0·1 grm. of iodine, and 0·5 of potassium iodide in 10 c.c. of normal sterile saline intraperitoneally.

Antitryptic titre	Before inoculation	4	8	12	24	30	48 hours after inoculation
Unit in ...	60	70	70	70	60	50	40 c.mm. of serum

Another rabbit received the same quantity of iodine and potassium iodide subcutaneously. The change in the antitryptic titre was the same.

Experiments 58 and 59.—Injection of hypertonic saline and normal saline intravenously or intraperitoneally: No effect on the antitryptic titre.

(d) *Influence of Temperature-raising Drugs: β-naphthylamine.*

Experiment 60.—A rabbit of 2,420 grm. received 3·5 c.c. of a 3 per cent. solution of β-naphthylamine. The temperature rose from 102·4° to 106·2° F. Pupils became dilated, breathing disturbances, &c.

Antitryptic titre	Before inoculation	30 mins.	2	5	24	48	72 hours after inoculation
Unit in ...	50	50	60	75	55	45	45 c.mm. of serum

Experiment 61.—A rabbit was inoculated with 10 c.c. of a 5 per cent. solution of calcium chloride intraperitoneally. Temperature fell from 102·8° to 96° F.

Antitryptic titre	Before inoculation	30 mins.	1	3	6	24	48	72 hours after inoculation
Unit ...	60	60	60	60	60	55	50	50 c.mm. of serum

(*c*) *Influence of Drugs damaging the White Corpuscle-forming Tissue.*

Experiment 62.—A rabbit received 1 c.c. of benzol subcutaneously every day for four days.

Antitryptic titre	Before inoculation of the first dose	6 hours	24 hours' inoculation second dose	30 hours	48 hours' inoculation third dose	72	96	99	102	105	120 hours after inoculation
Unit in ...	60	60	60	60	60	60	60	60	65	60	60 c.mm. of serum

Experiment 63.—A variation in antitryptic titre similar to that occurring with albumen occurs when a fat such as pure cod liver oil is inoculated intraperitoneally into a rabbit. A rabbit was inoculated with 10 c.c. of sterile pure oleum morrhuæ intraperitoneally.

Antitryptic titre	Before inoculation	3	6	24	48 hours after inoculation
Unit in ...	50	60	60	50	35 c.mm. of serum

(V) ANAPHYLOTOXIN. IN VITRO PRODUCTION.

In another paper [4] it has already been shown that the substances which are commonly used for the production of anaphylotoxin *in vitro* such as agar, starch, bacteria, do not cause adsorption of antitrypsin but cause adsorption of the proteolytic ferment that may be present.

Experiment 64.—The following examples of experiments show that a serum may become intensely toxic without any alteration in antitryptic titre or increase in non-coagulable nitrogen. In the agar experiments 0·5 per cent. of agar sol. was made in normal saline; it was kept at 56° C. till it was mixed with the serum which was at 37° C., the mixture was thoroughly shaken and then gelled on the ice before incubation.

Serum	Treatment	Antitryptic titre		Non-coagulable nitrogen		Fatal dose to guinea-pig
		Before	After	Before	After	
(*a*) 10 c.c. rabbit ...	+ 1·5 c.c. agar	60	60	3·54	3·54	3 c.c. 280 grm. pig
	Four hours 37° C.; sixteen hours room temperature.					
(*b*) 10 c.c. guinea-pig	+ 1 c.c. agar	20	20	4·5	4·5	2·5 c.c. 285 grm.
	Four hours 37° C.; sixteen hours room temperature.					
(*c*) 10 c.c. guinea-pig	+ 100 mgr. bacillus 80	25	25	3·8	3·85	5 c.c.; death in five hours; 190 grm.
	Four hours 37° C.; twelve hours room temperature.					
(*d*) 7 c.c. guinea-pig	+ 3 c.c. starch	30	30	3·6	3·6	5 c.c.; *nil*
7 c.c. guinea-pig	+ 1 c.c. 1 per cent. silicic acid	30	26	3·6	3·6	3 c.c.; convulsions, paresis, recovery
7 c.c guinea-pig	+ 1 c.c. 0·5 per cent. agar	30	26	3·6	3·6	3 c.c.; acute death
	Incubated four hours at 37° C., then inoculated, &c.					
(*e*) 10 c.c. guinea-pig	+ 2 slopes *Bacillus typhosus*	24	24	3·42	3·4	3 c.c.; acute death
	Incubated four hours at 37° C.					

Other experiments gave similar results.

From these experiments we can conclude—

(1) That in practically every case of bacterial infection there is a variation in the antitryptic index of the blood plasma—a negative phase varying in amount and duration, but usually representing a drop of 20 per cent. lasting on the average about eight hours, and a positive phase lasting from forty-eight to ninety-six hours.

(2) That the variation in the antitryptic index of the blood bears no relationship to the intensity of the infection or the toxicity of the protein inoculated: a first inoculation of egg albumen produces the same variations as a pathogenic bacterium, with equal weights of the various forms of protein. (Experiment 11.)

(3) That previous immunization appears to have practically no influence on the antitryptic curve produced by inoculation of the homologous antigen; similarly sensitization of the antigen produces no difference.

(4) That an infected animal may die with its antitryptic index in any phase: the index may be low if the death is rapid, high if slower; in this stage the blood and tissues may be swarming with bacteria and yet the index is high. On the other hand, an animal may recover with the index lower than its normal. (Experiment 20.) Again animals may die acutely and show no alteration in the titre. (Experiments 15 and 16.) And again an animal may die from an injection which has produced no initial negative phase in the titre (but only a rise). (Experiments 4, 23, 25.)

(5) That in anaphylactic shock the variations in the antitryptic index depend on the acuteness of the shock; in the fulminating shock there is no alteration, in the delayed there is a fall; and this is not on the average appreciably greater than with a first inoculation of a similar amount of antigen. With recovery from anaphylactic shock the index follows the same course as after a first injection.

(6) That anaphylotoxin can be prepared *in vitro* by substances or bacteria which cause no adsorption of antitrypsin, no auto-digestion such as can be demonstrated by an increase in the amount of non-coagulable nitrogen appears to take place. In fact, it has been shown that agar, &c., far from causing adsorption of antitrypsin and consequent auto-digestion, really hinder proteolytic degradation by adsorbing the ferment and not the anti-ferment.

(7) That food or a short period of abstinence from food has little influence on the antitryptic titre.

(8) That temperature-raising drugs like β-naphthylamine produce

a marked variation in the titre, but calcium salts, which also cause temperature variations, do not produce any change.

(9) That lactic acid produces a rapid and marked fall in the blood antitrypsin. Even when the fall equals 60 per cent. loss and lasts twenty-four hours, the animal appears to suffer no apparent ill-effect beyond the transitory marked dyspnœa directly after the inoculation. The animal, however, shows a great increase of non-coagulable nitrogen in the blood amounting to an increase of 50 per cent. in the first four hours. In infections there is no appreciable increase in non-coagulable nitrogen in the first four hours, in fact, in acute toxæmia the animal may die in four to six hours with no appreciable increase in the non-coagulable nitrogen. Then again the comparison between lactic acid and phosphorus poisoning is interesting. With lactic acid the antitryptic fall is rapid and prolonged and yet there are no signs of proteose poisoning, and the tissues show no gross change when the animal is killed some time after the inoculation; with phosphorus poisoning, the fall in antitrypsin only occurs shortly before death, but the blood has a great increase in non-coagulable nitrogen long before there is any fall in antitryptic titre [4].

(10) That although the antitryptic index varies especially after the inoculation of bacteria and protein substances, yet the inoculation of pure fatty substances produces the same type of variation.

The variations in the antitryptic index of the blood are important in view of Jobling's [5] theory of the causation of bacterial toxæmia, and anaphylotoxin production both *in vivo* and *in vitro*. Jobling like Vaughan [6] and Teale and Embleton [7] was impressed by the uniformity in the important symptoms of bacterial toxæmia in animals of the same species fatally infected with widely different types of bacteria. His observations led him to conclude that there was a common cause in all, that the symptoms of poisoning were all through caused by the formation and accumulation of toxic proteoses due, both in infection and anaphylactic shock, to auto-digestion of the plasma in the former, and with anaphylotoxin produced *in vitro* to the auto-digestion of the serum. His theory is that all these different conditions cause the adsorption of the antitrypsin and consequent auto-digestion of the serum or plasma.

(i) Dealing with anaphylotoxin produced *in vitro*. Our experiments show that an intensely toxic serum can be prepared and yet there is no evidence of any alteration in the antitryptic content or non-coagulable nitrogen of the serum used. Further they show, as seen in another paper by us [4], that far from the agar, &c., causing an adsorption of the antitrypsin and allowing unhindered activity of any proteolytic ferment, the agar hinders the activity of the ferment by adsorbing it. Hence, although the methods at our disposal for demonstrating the toxic higher albumoses in small quantity are very inadequate and their possible presence thus missed—the evidence on the whole does not appear to favour the idea that "anaphylotoxin" produced *in vitro* is due to proteolytic auto-digestion of the serum.

(ii) In acute anaphylactic shock there is no diminution of antitrypsin and in delayed shock the fall is not appreciably greater than with the primary inoculation. Hence if anaphylactic shock were solely due to proteose intoxication following adsorption of antitrypsin it ought to occur equally with the primary as with the second inoculation. Then again, if loss of antitrypsin is to account for the production of toxic proteoses, there must be local areas in which the antitrypsin must be completely adsorbed to allow of the action of the proteolytic enzyme —this might conceivably occur in the capillaries of the lungs and liver especially, where the bacteria and by analogy foreign protein may accumulate after injection. This would then mean that the adsorption of antitrypsin in these local areas is insufficient to make an appreciable difference in the antitrypsin of the blood as a whole. Jobling [8] further concludes that the rapid death following the intravascular inoculation of agar suspension or kaolin, &c., is due to the same cause—viz., the agar adsorbing the antitrypsin and permitting auto-proteose intoxication. These acute deaths however, as will be shown later, are due to intravascular clotting, &c., and are not accompanied by alteration in the antitryptic titre, and can be prevented by simultaneous or preceding injection of leech extract.

(iii) The question of the relation between antitrypsin and bacterial toxæmia next requires discussion. It has already been shown that the variations in the antitryptic curve show no relationship to the pathogenicity, toxicity or virulence of the bacteria or foreign protein inoculated. Hence it would appear that the toxin-provoking powers of a bacterium during infection are in no way parellel to the antitryptic fall, and this should be expected according to Jobling's theory if the toxicity is due to auto-proteose intoxication following antitrypsin adsorption by the inoculated bacteria.

Thus again for any auto-digestion of the plasma to occur we must suppose that there must be local areas of practically complete adsorption of the antitrypsin. This can conceivably only occur in the central organ capillaries where the bacteria rapidly become accumulated by becoming enmeshed in incipient clot prior to phagocytosis [9]. Hence if auto-degradation following removal of the antitrypsin inhibition is to occur at all it could only occur there, and the initial fall in the peripheral blood is due to this local removal of antitrypsin. Against this, in addition to the points mentioned above, are: (1) that a rapid fatal result can occur without any fall in the antitryptic titre; (2) that animals may die twenty-four to seventy-two hours after infection with a high antitryptic titre and in a septicæmic condition. The only possible explanation for this would be that the new production of antitrypsin provoked by the initial loss leads to over production, and thus the initial negative phase rapidly gives way to a marked positive phase.

These experiments show that an animal may die from septicæmic infection with the antitryptic titre 50 per cent. or more above normal, so that if auto-digestion is still to be invoked as the cause of the toxæmia it would still be necessary to postulate, forty-eight and even seventy-two hours after the primary infection, local areas of absent antitryptic inhibition unaffected by the general increase, which does not seem very probable. In the septicæmic condition such as in the secondary bacteriæmia of anthrax the phenomenon of the retention of the bacteria in the capillaries of the central organs and the consequent rapid clearing of the peripherally circulating blood does not occur—in the secondary bacteriæmia of this septicæmic state the bacteria are fairly evenly distributed so that, if the bacteria cause adsorption of the antitrypsin at all, there ought to be a rapid fall in titre with its occurrence. This bacteriæmia occurs rapidly and one would hardly expect a still further new rapid production to occur in this state to make up for the loss. There thus seems to be no relation between antitryptic titre and intensity of toxæmia. There thus seems to be no support for the theory of auto-proteose intoxication brought about in this way, especially as bacteria do not adsorb antitrypsin *in vitro*.

(iv) What is the reason of the variation in the antitryptic curve?

(1) Some experiments by us to be published soon, show that an animal may die in the first four hours after inoculation of a culture of bacteria without any evidence of proteoses in the blood or any increase of N.C.N.,[1] later the increase occurs rapidly and in marked degree, and is almost wholly due to urea accumulating in the blood. This increase is all the more marked since badly infected animals take no

---

[1] N.C.N. = Non-coagulable nitrogen.

food and in normal animals abstinence from food greatly diminishes the amount of N.C.N. in the blood. The evidence, therefore, points to greatly increased tissue katabolism, so that the inference is that the serum antitrypsin may have an inhibiting effect on tissue autolysis; but our experiments [4] on this subject show that tissue autolysis is in no way influenced by serum antitrypsin except in so far as the serum alters the amount of free H ions on which the autolysis depends.

(2) The possibility of the antitrypsin having to do with the inhibition of leucocytic ferments which might possibly be liberated during phagolysis. *In vitro* experiments show that serum antitrypsin influences leucocyte autolysis. In infection, however, the antitrypsin variation occurs early—in fact too early to be explained by a neutralization of liberated leucocytic ferments due to phagolysis, and further the same occurs when apparently absolutely innocuous proteins are inoculated primarily.

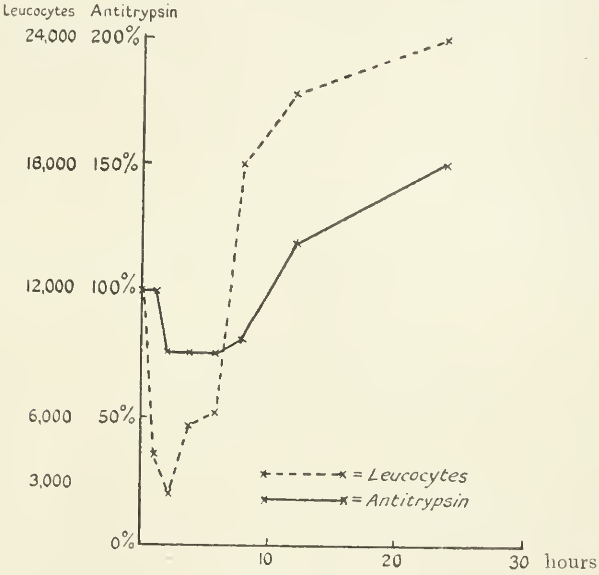


CHART 1.

Chart showing relationship between antitryptic titre and the leucocyte count in a rabbit inoculated with half a twenty-four hour agar slope of *Bacillus typhosus*. This relationship is by no means universal, since as has been shown above, the antitryptic titre may not fall or even go up but the leukopænia apparently always occurs.

The only fact is that the antitryptic curve varies to some extent with the leucocyte count, with the initial leucopenia it falls, with the subsequent leucocytosis it goes up, but there is no marked degree of relationship of the curves.

The antitrypsin is apparently not liberated from the leucocytes during clotting of the blood—it is present in the same amount in the plasma as the serum, and the amount is not diminished by the disintegration of the leucocytes during clotting.

(VI) Influence of Antitrypsin on the Growth of Bacteria.

(6) We finally have to consider the point raised by Wright. Wright states that certain bacteria can only grow in the animal body and produce pathogenic effects if the split products of protein digestion are ready at hand for them. He believes from his experiments that this occurs when the serum antitrypsin is destroyed, and the inhibition is therefore removed from the autolytic ferments. Bearing on this we have to note the following facts: (*a*) That serum antitrypsin is only inhibited when the reaction is N/20 acid (lactic acid) and the serum proteolytic ferment is destroyed by a much lower H ion concentration [4]. (*b*) That serum antitrypsin in no way affects tissue autolysis except as mentioned above in virtue of the serum neutralizing and diminishing the H ion concentration [4]. (*c*) That the plasma or serum autolytic ferment is a very weak one. (*d*) That the autolytic ferments of the tissues are very sensitive to increased H ion concentration [4].

Thus we would conclude that the split products necessary for the vitality of this special class of bacteria in the tissues are not derived from the serum or tissues because of the inhibition of serum antitrypsin, but are derived from the autolytic degradation of the tissues when the tissues locally are so much damaged that the tissue fluids there are no longer capable of neutralizing the H ion present, and in proportion to the concentration of these H ions autolytic degradation occurs. The viability of the organisms in no way depends on the removal of the inhibitory power of serum antitrypsin. These organisms can derive all the food they require from the tissues, which can autolyse even in the presence of functionally active antitrypsin.

Further, Wright's [10] experiments *re* the growth of *Bacillus Welchi* in blood serum do not show the necessary relationships postulated by him—viz., sufficient acidity to destroy the antitrypsin, and thus activate the proteolytic ferment. In his *in vitro* experiments it can be seen that the vigorous growth occurs with a degree of acidity insufficient to inhibit antitrypsin and yet capable of destroying the proteolytic ferment. Then again, Wright's method of demonstrating increased activity of growth of certain bacteria when the antitrypsin is "neutralized" by the addition of definite amounts of trypsin is inaccurate, and quite different results can be obtained if the serum antitrypsin is inhibited by other methods. Now neutralization of antitrypsin by trypsin is not a true one; it is only temporary, is incomplete, and lasts until the antitrypsin has been digested by the trypsin. Antitrypsin is purely protein in nature. Thus split products are produced during the course of the experiment from this source alone even if the amount of trypsin added is insufficient to act upon the rest of the substrate during the course of the whole experiment. Wright's method of estimating the amount of trypsin necessary to inhibit the antitrypsin is determined by a preliminary experiment of shorter duration than that in which the bacterial activity is determined. Hence the amount of trypsin added is such that the antitrypsin is capable of inhibiting the action of the trypsin on the rest of the substrate for only a short time, and for the remainder of the duration of the experiment the trypsin, having destroyed the antitrypsin, acts upon the substrate, producing an abundance of split products on which the bacteria grow readily.

Experiment 66.—Some rabbit serum was taken and divided into two parts: (*a*) untreated, (*b*) treated with chloroform at room temperature for three hours to destroy the antitrypsin, evaporated to dryness *in vacuo* to remove the chloroform and made up in sterile saline to original volume; 10 c.c. of each was tubed and inoculated with one drop of an emulsion of *Bacillus Welchi* from an agar slope growth. Each was incubated anaërobically and then tested by plating out in duplicate one drop of the culture. Each test was made in duplicate, and the figures given represent an average of the counts.

	(*a*) Untreated serum	(*b*) Treated serum
Without incubation ...	82 colonies	77 colonies
After twenty-four hours ...	54 ,, ...	94 ,,
After forty-eight hours ...	120 ,, ...	201 ,,

Other experiments gave similar results.

Hence the removal of serum antitrypsin has very little influence on the rate of growth of *Bacillus Welchi* in the serum. In the tissues the primary factor is the reaction, and this with the consequent autolysis favours the growth of this class of organism. The serum antitrypsin plays no inhibitory part, the only inhibitory action exercised is in proportion to its alkalinity.

CONCLUSIONS.

In this paper the variations in antitrypsin in infection and anaphylactic shock are recorded.

There appears to be no relationship between the antitryptic curve and the intensity of the infection, pathogenicity or virulence of the infecting organism.

There is also no relation between the curve and the toxicity of a bacterium to the infected animal.

These experiments negative the view taken by Jobling that the toxæmia resulting from bacterial infection is due to the formation of toxic proteoses subsequent to the removal of the antitrypsin inhibition on the plasma autolytic ferments.

Further, Wright's hypothesis of the viability and consequent infectivity of certain organisms being due to the removal of the antitrypsin inhibition with the consequent formation of protein degradation bodies for their growth is negatived; the degradation bodies are apparently formed from autolysis of the tissues as the result of the increase of the H ion concentration at the site of infection.

REFERENCES.

[1] TEALE and BACH. "Nature of Serum Antitrypsin," *Proc. Roy. Soc. Med.*, 1920, xiii (Sect. Path.), p. 1 *et seq.*
[2] TEALE and BACH. *Ibid.*
[3] TEALE and EMBLETON. *Lancet*, 1913, i, p. 236.
[4] TEALE and BACH. *Proc. Roy. Soc. Med.*, 1920, xiii (Sect. Path.), p. 33.
[5] JOBLING, &c. *Journ. Exp. Med.*, 1914, xix, p. 480, xx, p. 37.
[6] VAUGHAN. *Journ. Amer. Med. Assoc.*, 1914, lxii, p. 583.
[7] TEALE and EMBLETON. *Lancet*, 1913, i, p. 237.
[8] JOBLING. *Journ. Exp. Med.*, 1915, xxii, p. 590.
[9] TEALE and BACH. "Mechanism of Removal of Bacteria from the Circulation" (in the Press).
[10] WRIGHT. *Lancet*, 1917, i, p. 6; *Proc. Roy. Soc. Med.*, 1917, x, p. 1.

JOHN BALE, SONS & DANIELSSON, LTD., 83-91, Great Titchfield Street, London, W.1.

Th. 9

[illegible]

Journal of Pathology and Bacteriology, Vol. XXIII.

STUDIES IN INFECTION—II.

THE PATHS OF SPREAD OF BACTERIAL EXOTOXINS WITH SPECIAL REFERENCE TO TETANUS TOXIN.[1]

By F. H. Teale and Dennis Embleton.

From the Research Laboratories, University College Hospital Medical School, London.

SECTION I.

SPREAD OF TETANUS TOXIN.

A. Subcutaneous Inoculation.

B. Intravenous Inoculation.

  1. Passage into the Tissues.
  2. „ „ Cerebro-spinal Fluid.
  3. „ „ Central Nervous System.

C. Neural Spread.

Axis Cylinders v. *Neural Lymphatics.*

  1. Lymphatic spread of Washed Spores.
  2. Blocking of Lymphatic Channels.
  3. Channels of Ascent of Colloidal Dyes.
  4. Effect of Section of Ventral Nerve Roots.

D. Action of Iodine on Tetanus Toxin.

E. Site of Action of Tetanus Antitoxin.

  1. Passage to Central Nervous System by Blood Stream.
  2. Passage from Cerebro-spinal Fluid.
  3. Passage along Nerves.

This paper is a continuation of a study on bacterial invasion and infection, the first part of which was published in the *Proceedings of the Royal Society of Medicine* (1914 [1]). In it we consider the paths of spread of the exotoxins, especially tetanus toxin, and, in view of the importance of the subject, the site of action of tetanus antitoxin.

At first sight it may appear to have been superfluous to undertake the study in view of the work of Meyer and Ransome (1903 [2]). These observers state that their experiments lead them to conclude that tetanus toxin reaches the central nervous system solely by way of the axis cylinders of the motor nerves, and that if it ascends by the sensory nerves towards the cord the posterior root ganglion acts as a block to further spread, since tetanus dolorosus only develops when the poison is experimentally inoculated into the posterior root between the ganglion and the cord. They came to the conclusion that the toxin did not spread to the cord by way of the perineural lymphatic sheaths.

---

[1] [Received May 8, 1919.] The expenses of this research were defrayed by a grant from the Medical Research Committee.

Orr and Rows (1916[3]) showed that if bacteria were left in contact with a nerve trunk they could be demonstrated in the spinal cord, having travelled along the perineural lymphatic sheath along the posterior root to the cord.

Golla (1917[4]), who carried out some experiments for the Tetanus Committee of the War Office, was also inclined to believe in some degree in the perineural lymphatic spread.

Marie and Morax (1902[5]) concluded that the toxin only ascended by way of the axis cylinders. They found that after section of a nerve tetanus toxin could still be absorbed by the peripheral end, but much more slowly, and this occurred even two days after the section had been performed. Later it did not occur, this coinciding with the occurrence of degeneration of the axis cylinder.

Before entering into these points it is necessary first to record our experiments with regard to the distribution of tetanus toxin after subcutaneous and intravenous inoculation.

A. Results of Subcutaneous Inoculation with Tetanus Toxin.

Experiment 1.—Rabbit of 1850 grms. received 10 c.c. of a potent tetanus toxin subcutaneously into the right hind-leg. The animal was killed after eight hours, and, after thorough washing out with normal saline, emulsions were made of its tissues, and 0·1 grm. of each tissue was inoculated into mice. The cord was cut into segments corresponding to the lumbar nerve roots, and each piece, ground up in a small quantity of saline, used for inoculation. The sciatic nerves were divided at the level of the knee into upper and lower fragments. The upper fragment included the roots. The rabbit was also bled from the ear at intervals to ascertain the rate of appearance of the toxin in the blood.

1 c.c. serum, 2½ hours after inoculation	Nil.
1 ,, ,, 6½ ,, ,,	Nil.
1 ,, ,, 8 ,, ,,	Died of tetanus in 5 days; local symptoms after 4 days.
Lower end right sciatic (side inoculated)	Died in 48 hours.
Upper end ,,	Nil.
Left sciatic	Nil.
Segments of cord	Nil.
Right popliteal gland	Death in 40 hours.
Spleen	Symptoms in 7 days; died 8th day; typical symptoms.
Liver	Nil.
Marrow	Nil.
Lung	Nil.
Chyle (0·5 c.c.)	Death in 3 days.
Urine	Nil.

Thus the toxin was present in the blood serum in 8 hours in quantity sufficient for 1 c.c. to contain a M.L.D. for a mouse. The toxin had not travelled far up the sciatic nerve, but had got through the lymphatic glands to the chyle and blood stream.

Experiment 2 (like Experiment 1).—Rabbit, 2000 grms., 10 c.c. of the same tetanus toxin inoculated subcutaneously into the right leg. Animal killed 12 hours after inoculation. Inoculations into mice as before.

Serum, 1 c.c.	Death in 36 hours.
Œdema fluid from seat of inoculation	,, 12 ,,
Popliteal gland	,, 21 ,,
Pelvic gland	,, 24 ,,
Lumbar gland	,, 24 ,,
Chyle (1 c.c.)	,, 22 ,,
Lower end of right sciatic nerve	,, 24 ,,
Upper end	,, 34 ,,
Sciatic nerve, left	Nil.
Spleen	(Died : septic).
Marrow	Death in 96 hours.
Liver	Symptoms local tetanus 6th day.
Brain	Nil.

Here several lethal doses were present in the quantities of serum, chyle, nerve, etc., used, but very little in the other viscera.

Experiment 3.—Rabbit of 2000 grms. Same dose as Experiment 2, also inoculated subcutaneously into right leg. Kept alive for 25 hours. Symptoms of tetanus.

1 c.c. serum	Symptoms after 96 hours.
1 ,, chyle	,, 48 ,,
Cerebro-spinal fluid	Nil.
Seat of inoculation	Died in 72 hours.
Popliteal gland	Died immediately (accident).
Pelvic gland	Died in 24 hours.
Lumbar gland	,,
Spleen	,,
Liver	Nil.
Marrow	24 hours.
Lower right sciatic	Died in 24 hours.
Upper ,,	,, 36 ,,
Left sciatic (upper)	,, 48 ,,
,, (lower)	Nil.
Cord segments	Nil.
Medulla	Nil.
Brain	Nil.

Here the results are as in Experiment 2, only there is a greater accumulation of toxin in the spleen, marrow, and nerve of the side inoculated, and in addition there is toxin in the opposite sciatic nerve. Further, these results prove that the presence of the toxin in the tissues like the spleen, marrow, etc., in this experiment, is not due to insufficient washing out, since 0·1 grm. of the tissues killed in 24 hours, whereas the serum in doses of 1 c.c. only produced symptoms in 96 hours, hence the result of the tissue inoculations cannot be due to retained blood. The same can be seen in the next experiment.

Experiment 4.—Same as previous experiment. Subcutaneous inoculation into right leg. Animal kept alive for 48 hours, marked symptoms of tetanus.

1 c.c. serum	Nil.
Seat of inoculation	Died 7th day.
Popliteal gland	,, 5th ,,
Pelvic ,,	,, 4th ,,
Lumbar ,,	,, 4th ,,
Chyle (0·5 c.c.)	Nil.

Spleen	Died in 96 hours.
Liver	Nil.
Marrow	Nil.
Lower right sciatic nerve	Died in 48 hours.
Upper ,, ,, ,,	do.
Left sciatic (upper end)	Local tetanus, 4th day.
Brachial plexus	Nil.
Cord segments	Nil.

In 48 hours, the serum in quantities of 1 c.c. no longer contained enough toxin to give rise to even local tetanus. The lymphatic glands also had very little. The same was the case with the spleen. The other viscera apparently contained no toxin. There was, however, a small amount of toxin in the opposite sciatic nerve.

Experiment 5.—A cat was anæsthetised with ether, inoculated with 10 c.c. of tetanus toxin subcutaneously into the right leg. The thoracic duct was exposed and the chyle collected.

	Result.
First quarter of an hour; inoculated into mouse	Nil.
Second ,, ,, ,, ,,	Nil.
Third ,, ,, ,, ,,	Typical local tetanus in 50 hours: death, 96 hours.

The animal was killed. *Post-mortem.*—There was no apparent damage to vessels, capillaries, etc., at the seat of inoculation. This experiment shows that the toxin passes rapidly through the lymphatics to the blood stream.

These experiments thus confirm those of Marie and Morax (1902 [5]) and Meyer and Ransome (1903 [2]). They show—

1. That tetanus toxin is present in the nerve of the side injected. The toxin may, however, not only be present in the axis cylinders, but also in the perineural and epineural lymphatics. It seems possible to us that it is only the toxin in the latter position that can be demonstrated by inoculation into mice, since it is possible that just as the tissues of the central nervous system render the toxin inert, as shown by the mouse test, so the axis cylinders and medullary sheaths of a peripheral nerve may do the same for any toxin ascending in them.

2. Although the toxin is present in the central nervous system, as evidenced by the symptoms, it cannot be demonstrated when the tissue is inoculated into a mouse.

3. The toxin inoculated into the subcutaneous tissues spreads along the lymphatics to the glands, thence to the thoracic duct into the circulation, and is taken up to some small extent by the marrow, spleen, liver, etc., just as is the case with bacteria, and as will be shown later for diphtheria toxin. This is in opposition to the results of Bruschettini ([6]), quoted by Meyer and Ransome.

4. The experiments also bring out the slight spread of the toxin down the nerve of the opposite limb. This cannot be explained at all easily if the toxin is believed to spread solely along axis cylinders, but if it also spreads along the neural lymphatics to the lymphatic

spaces of the cord and thence to the opposite nerve lymphatic spaces, the path is obvious.

B. RESULTS OF INTRAVENOUS INOCULATION WITH TETANUS TOXIN.

1. *Rate of Appearance of the Toxin in the Chyle.*

Experiment 7.—A large cat was fed on milk to get a good flow of fatty chyle. It was then inoculated intravenously with 50 c.c. of tetanus toxin. The thoracic duct was exposed, and the chyle collected and injected into mice.

¼	c.c.	collected in first	5	minutes	Nil.
¼	,,	,,	5–10	,,	Died in 72 hours.
¼	,,	,,	10–15	,,	,, 40 ,,
¾	,,	,,	15–25	,,	,, 22 ,,
⅘	,,	,,	25–30	,,	,, 18 ,,
⅕	,,	,,	30–40	,,	,, 18 ,,
⅖	,,	,,	40–45	,,	,, 18 ,,
½	,,	,,	45–60	,,	,, 18 ,,
¼	,,	,,	in second hour after inoculation		,, 18 ,,
2	,, serum	,,	,, ,, ,,		Local tetanus on 4th day. The mouse was killed on the 6th day, no generalisation.

This shows that the tetanus toxin has such a small molecule that it rapidly passes from the capillaries into the connective-tissue spaces and thence into the thoracic duct. The chyle secreted during the second five minutes contained several lethal doses for a mouse. A foreign serum injected intravenously cannot be detected in the chyle even after several hours, as will be seen from the following experiments.

Experiment 8.—A cat was anæsthetised and inoculated intravenously with 20 c.c. of horse serum, the thoracic duct was opened and the chyle collected for 3 hours, and then inoculated subcutaneously into guinea-pigs :—

1. Chyle of first hour.
2. ,, ,, second hour.
3. ,, ,, third hour.

After three weeks the animals were tested for hyper-sensitiveness against horse serum, with negative results.

Experiment 9.—Some horse serum was iodised and was tested to be certain that there was no free iodine, 10 c.c. were then inoculated intravenously into a cat under anæsthesia. The thoracic duct was opened and the chyle collected for 3 hours.

The chyle was then treated with acid to liberate any iodine and then tested. The result was negative.

2. *After Intravenous Inoculation the Toxin cannot be demonstrated in the Cerebro-spinal Fluid.*

Experiment 10.—A cat was anæsthetised, the left jugular vein was exposed, and 20 c.c. of tetanus toxin were inoculated into it. The sub-arachnoid space was exposed in the region of the sub-cerebellar space and the cerebro-spinal fluid collected for 2 hours. The animal was finally inverted to obtain all the fluid possible from the spinal canal. The fluid was injected into mice.

1 c.c. collected in first 30 minutes	Nil.
1 ,, ,, second 30 ,, ...	Nil.
1½ ,, ,, ,, 60 ,, ...	Nil.

The animal was killed, the choroid plexus removed, ground up, and injected into a mouse without result, 1 c.c. of the cat's serum killed a mouse in 18 hours. In another experiment the vessels were damaged, and so the fluid was blood-stained, with the result that toxin passed into it. Specimens of cerebro-spinal fluid obtained by one of us from cases of tetanus also produced no results in mice, even after concentration *in vacuo*.

These experiments prove that the tetanus toxin is not allowed to pass into the cerebro-spinal fluid.

3. *The Question then arises if Tetanus Toxin can pass directly from the Capillaries into the Tissues of the Central Nervous System.*

We have already mentioned that tetanus toxin which has gained access to brain tissue cannot be demonstrated by subcutaneous inoculation of the tissue into a mouse. Metchnikoff (1898[7]) has, however, drawn attention to the fact that in the guinea-pig subcutaneous inoculation of tetanus toxin exposed to brain tissue *in vitro*, was followed by tetanus. His explanation is that, owing to the slowness of the phagocytosis of the brain, toxin emulsion in the subcutaneous tissues of the guinea-pig, the toxin became liberated from its combination and was able to produce symptoms.

Experiment 11.—A rabbit was inoculated with 20 c.c. of tetanus toxin intravenously. The animal was kept alive for 24 hours, when it had marked symptoms of cephalic tetanus. It was then killed and washed out with normal saline. The brain and cord were ground up and inoculated subcutaneously into two guinea-pigs without result.

Now if the toxin could gain direct access to the central nervous system from the capillaries, the symptoms of cerebral tetanus, just like those produced experimentally by direct intracerebral inoculation, should be expected to occur. These symptoms, however, never occur. Intravenous inoculation is followed by the symptoms of cephalic tetanus, due to the spread of the toxin along the cephalic nerves, the centres linked up with the periphery by the shortest nerve paths showing symptoms first.

These observations show that the capillaries of the central nervous system and choroid plexus have a selective and thus a protective action and do not allow tetanus toxin to pass through them as do the tissue capillaries. The same result is noted if potassium ferrocyanide be inoculated intravenously. In such an experiment the iron salt can be demonstrated in the chyle in the first five minutes after the intravenous inoculation, in the connective tissues, organs, etc., and is rapidly eliminated by the kidneys. The central nervous system shows no increase in iron even by the most delicate method of demonstrating the iron by means of the hydrazine sulphate and dimethyl glyoxine test (shows 1 : 100,000,000). The choroid plexus, however, gives a very marked iron reaction.

Also when potassium ferrocyanide is inoculated into the subcutaneous tissue its presence can be demonstrated in the nerve supplying the seat of inoculation up to the corresponding segment of the cord and even in the opposite nerve.

Experiment 12.—This experiment was performed by inoculating 5 c.c. of a 10 per cent. solution of potassium ferrocyanide into the right hind-paw of a rabbit, and leaving it for twenty-four hours. The animal was then killed, bled, washed out with iron-free normal saline, the sciatic nerves and cord divided up in the usual way, the organic material destroyed, and the test for iron performed with the method given above, the results being compared with the same tissues from a normal animal. Depth of pink indicated by + + +, + +, +, –, (– = practically colourless).

	ANIMAL EXPERIMENTED ON.	CONTROL.
Seat of inoculation	–	–
Popliteal gland	–	+ (hæmorrhage).
Lumbar gland	+	–
Serum	–	–
Lower right sciatic	+	–
Upper ,, ...	+ + +	–
Left sciatic (upper)	+ +	–
,, (lower)	–	–
Lower lumbar cord and plexus . .	+ + +	–
Upper ,, ,, . .	+	–
Dorsal ,, ,, . .	–	–
Brain ,, ,, . .	+ +	+ +

C. THE QUESTION OF THE SPREAD ALONG THE NEURAL LYMPHATIC CHANNELS.

In the first section (A) we confirmed the observations of Meyer and Ransome (1916 [2]) that the toxin was present in the nerve trunks, but could not state definitely that it was only in the axis cylinders. Some of our experiments were suggested by the results obtained by Orr and Rows (1916 [3]), who found that bacteria left in contact with a nerve trunk ascended by the neural lymphatics to the cord and could be found spreading along the posterior nerve roots through the spinal ganglia to the cord.

1. *Experiments using Washed Spores for Injection into the Nerve Trunk.*

In these experiments we made use of an observation made by us that when washed spores are inoculated into an animal the spores do not remain quiescent, but that a certain number develop into vegetative forms, remain unphagocyted for a long time, only becoming gradually destroyed by phagocytosis in the tissues, and do not appear to multiply. We accordingly took some cultures of *B. anthracis* which had well spored and heated a saline emulsion of the cultures at 80° C. for 3 hours. These washed spores germinated when sown on nutrient media, and on inoculation into a guinea-pig the cultures thus obtained were typically pathogenic. Some of the washed spores inoculated directly into the peritoneal cavity, followed next day by a small subcutaneous dose of lactic acid, proved fatal, whereas the washed spores by themselves in similar or even larger doses, in spite of germination, did not produce disease.

Experiment 13.—0·25 c.c. of a very thick emulsion of anthrax spores so treated was inoculated into the upper part of the sciatic nerve of a series of rabbits, at a level just below the great trochanter. These animals, which never developed any symptoms of infection, were killed at intervals. In all there was some inflammatory reaction around the seat of inoculation, but the nerve roots, meninges, and cord showed no thickening or inflammatory reaction, and nowhere in the cord was there a leucocytic infiltration or glial proliferation. In specimens from a rabbit killed forty-eight hours after the injection, the vegetative forms of *B. anthracis* were seen in both the motor and sensory parts of the nerve, spreading in the case of the afferent nerve to the posterior root and through the spinal ganglion to the cord. The bacteria in their vegetative state were lying around the nerve fibres. Similarly, they spread along the efferent part of the nerve to the anterior root to the cord, and in each case extended well into the white matter but not into the grey. Later the bacteria could be traced around the anterior cornual cells.

These experiments thus confirm those of Orr and Rows (1916 [3]). They also suggest that if bacteria can spread along the neural lymphatic sheaths of both the efferent and afferent parts of the nerve trunk, and in the case of the latter through the spinal ganglion, and thus reach the cord, there is a strong *a priori* ground for inferring that tetanus toxin with its small molecule might do the same, in addition perhaps to ascending directly to the anterior cornual cell by the axis cylinder of the efferent nerve. This ascent along the neural lymphatic spaces to the lymphatic spaces of the cord also seems to be the most probable explanation of the route of spread of tetanus toxin along the cord and the sequence of muscular involvement.

In by far the greater number of rabbits experimented upon with tetanus toxin, the march of symptoms was homolaterally upwards from the injected limb cephalically, and it appears to us this can only be explained by cord lymphatic spread. In a few cases the opposite corresponding limb became affected next before the homolateral anterior when the injection was made into the hind-limb.

Later, of course, during an infection or from a large amount of toxin experimentally inoculated, the toxin which has got into the general circulation will ascend to the cord, etc., along the other nerves. Then the cephalic and trunk symptoms occur quickly, the toxin travelling along the shortest nerve trunks reaching the corresponding parts of the central nervous system quickest.

But at the seat of infection or inoculation, where the concentration of the toxin is greatest, more is available to ascend the local neural route to the cord than elsewhere, and so the spread locally from this to the neighbouring parts of the cord will take place more rapidly. The symptoms from this will be also more intense than those occurring in the other parts from the greatly diluted toxin ascending to them. If we believe that the spread is along axis cylinders only, it is impossible in our present state of knowledge of the central nervous system to explain the homolateral spread.

2. *In the Next Series of Experiments the Perineural Lymphatic Sheath was blocked in various ways to study any possible Alterations in the Onset of the Symptoms produced by Subcutaneous or Intraneural Inoculation of the Toxin distally to the Block.*

Experiment 14.—A rabbit was anæsthetised with ether. The left sciatic nerve was exposed with aseptic precautions midway between the popliteal space and the exit of the nerve from the spinal canal. Tincture of iodine was inoculated thoroughly into the nerve bundles. The wound was stitched up. The animal recovered and showed no sign of paralysis. After 7 days 5 c.c. of tetanus toxin were inoculated into each hind-leg. On the side which had not been treated with iodine tetanus developed in 28 hours; on the side which had been treated with iodine, the symptoms of local tetanus developed only after 50 hours, and were complete in 72 hours, when the animal was killed.

Experiment 15.—The same operation as Experiment 14 was performed on the right sciatic nerve of a rabbit with the same precautions, under ether; 14 days afterwards 2·5 c.c. of tetanus toxin were inoculated into the tissues of the hind-leg on the operated side. 2·5 c.c. of toxin were similarly inoculated into the hind-leg of a normal control rabbit. The normal rabbit showed marked tetanus of the inoculated leg in 36 hours, and later the symptoms spread on the homolateral side to the fore-limb in 48 hours.

In the iodine-treated rabbit, symptoms of tetanus, slight stiffness of the leg, occurred in 72 hours, and the rigidity was complete in 96 hours, but there was no spread. The animal was kept alive for 7 days.

Experiment 16.—Experiment on a rabbit under ether, aseptic precautions. The right sciatic nerve was injected with tincture of iodine as before, and 3 days later 5 c.c. of tetanus toxin were inoculated into each leg. In 24 hours the left leg was perfectly rigid, the right quite normal; in 48 hours the right also became affected.

These experiments show that the ascent of the tetanus toxin can be delayed by injecting iodine into the tissues of the nerve. This may presumably be supposed to act chiefly by causing inflammation and occlusion of the neural lymphatics, but may also cause some damage in the axis cylinders and so delay the ascent of the toxin up the axis cylinders. The damage, however, can only be slight, as there was no appreciable physiological damage of function in these animals and not the least apparent sign of weakness in the limb operated on. The possibility of the action of the iodine in the toxin will be discussed later.

Experiment 17.—A rabbit was anæsthetised, the right sciatic nerve exposed in the middle of the thigh, and ½ c.c. of egg albumen was injected into the nerve bundles. Then 2·5 c.c. of tetanus toxin were inoculated into the subcutaneous tissues of the hind-paw of the same side.

A control rabbit of the same weight was also given 2·5 c.c. of the toxin, into the right hind-paw. This latter rabbit had typical local symptoms in 24 hours, and complete rigidity of the whole extremity in 30 hours. The former rabbit showed no signs for 24 hours, and then some stiffness of the leg developed which lasted for 3 days and then passed off.

Experiment 18.—A rabbit was anæsthetised with ether and the sciatic nerves were exposed. Into the right sciatic was inoculated some normal horse serum, into the opposite some normal rabbit serum. Then into each hind-paw were inoculated 2·5 c.c. of tetanus toxin. Typical tetanus developed on the left side in 24 hours. On the opposite side nothing occurred till 72 hours, and the limb was completely rigid in 96 hours.

Experiment 19.—A rabbit was anæsthetised with ether; both sciatic nerves were exposed in the space between the great trochanter and the exit from the great sciatic notch.

Into the right nerve was inoculated ¼ c.c. of egg albumen, into the other ¼ c.c. of normal saline. Then into each sciatic nerve at a level just above the popliteal space, ¼ c.c. of concentrating tetanus toxin was inoculated. The wounds were treated aseptically and sewn up. There was no weakness in either limb.

In 24 hours the left leg had developed typical tetanus paralysis, the right was quite normal, and continued so for 3 days when the animal was killed.

A similar experiment gave the same result.

These experiments show—

1. That inflammatory occlusion of the neural lymphatic sheath greatly delays the onset and diminishes the severity of the symptoms of tetanus, due to the local inoculation of tetanus toxin. This might be supposed to be due to the action of the iodine on the toxin, but the interval allowed after the inoculation of the iodine we think excludes this supposition as do the other experiments. The question of damage to the axis cylinders has been discussed.

2. That blocking the lymphatic channel by a viscid colloid like egg albumen or normal horse serum does the same. Here inflammatory reaction in the axis cylinders cannot account for this delay, since normal rabbit serum, from another animal, similarly injected did not afford protection, and presumably the mechanical inflammatory reaction of the operation, etc., would be the same as with horse serum or egg albumen.

The neural lymphatic path thus appears to play some part in the spread upwards of the toxin, unless it is believed that in these experiments the inflammatory reaction around the seat of inoculation influenced the flow up the axis cylinders, but no physiological defect of motor control was apparent.

The question then arises, Why does the subcutaneous inoculation of tetanus toxin not produce tetanus dolorosus as well as motor tetanus?

Is it that the posterior root ganglion acts as a toxin filter, as suggested by Homen (1905 [8]) for bacteria, and keeps back the toxin from the posterior root zone and destroys it? Or is it that, in the experimental production of tetanus dolorosus, the cord gets damaged slightly in some way and that this is necessary for its production and that tetanus toxin reaching the unimpaired posterior root zones, etc., has no effect on the sensory channels? For one must conclude that if bacteria can reach the posterior root the bacterial trap of the posterior root ganglion must be only capable of acting to a limited degree. It might be argued that the spread is due to the bacteria, which have been caught in the ganglion, being sufficiently virulent to multiply and so break down the defence. At first sight this might appear to be the explanation, because if colloidal dyes like trypan-blue be inoculated into a nerve trunk the posterior root ganglion acts as a block to the further passage of the pigment, whilst its spread along the ventral root to the surface of the cord (*vide infra*) is unhindered.

But in the case of our experiments with washed spores of *B. anthracis* the element of multiplication of the bacteria does not arise, because we found experimentally that the vegetative forms of the bacteria which develop from the washed spores do not undergo multiplication but gradually die off. Hence we must conclude that if large non-motile bacteria can pass through the posterior root ganglion and reach the cord, it is highly probable that tetanus toxin might be able to do the same. Then how is it that tetanus dolorosus does not occur? It may be—

1. That the main lymphatic channels are along the anterior roots and little gets to the posterior root ganglion and is dealt with there.

2. That to produce tetanus dolorosus a lesion of the cord is necessary.

3. *Injection of Colloidal Dye into the Nerve Trunk.*

Experiment 20.—A rabbit was anæsthetised with ether, the left sciatic nerve exposed, and 0·2 c.c. of a thick suspension of trypan-blue in normal saline was inoculated into the nerve soon after its exit from the great sciatic foramen.

After 24 hours the rabbit was killed, and it was seen that the dye had extended along the perineural and epineural lymphatic sheaths as far as the posterior root ganglion; here the pigment ceased abruptly and the ganglion showed no pigmentation. Along the ventral root the pigment could be traced with decreasing intensity, and finally it appeared to diffuse itself faintly over the surface of the cord at the entry of the nerve root. There was no pigment in the cerebro-spinal fluid. Some of the pigment travelled along a branch lymphatic to the large gland at the sacral promontory. Other experiments gave similar results.

Experiment 21.—Same as above, except that the pigment was inoculated into the nerve in the mid-thigh region. The animal was kept alive for 12 hours. In this case not only did the pigment travel along the perineural and epineural lymphatic sheaths as in the previous experiment, but the axis cylinders were pigmented right up to the intervertebral foramina, the pigmentation being less deep there than lower down. The pigmentation of the axis cylinders was much more intense than that of the neural lymphatic sheaths, and sections showed the staining microscopically.

We obtained a similar result in experiments quoted in our previous paper (1914 [1]), p. 20, lasting only 1 and 3 hours, after injection.

Hence colloidal dyes pass up nerves both by way of the axis cylinders and lymphatic sheaths, but are stopped by the posterior root ganglion. Hence there is a marked difference between the spread of bacteria and colloidal pigments along the afferent nerve at the spinal ganglion. In these experiments the bacteria were non-motile and did not have the power of multiplication or causing inflammatory reaction, and for these reasons breaking down the defensive barrier that apparently exists to the colloidal dye.

Is tetanus toxin treated like the dye? To elucidate this the following experiments were performed.

4. *Section of Ventral Nerve Roots.*

Experiment 22.—A cat was anæsthetised, the lumbar cord was exposed and the ventral roots of the sacral and lumbar nerves cut so that there was complete paralysis of the hind-limb of one side. The wound was then sewn up. The whole operation was carried out aseptically. Then the sciatic nerve of the same side was exposed as high as possible, the wound and nerve were carefully packed round with cotton-wool, and 0·35 c.c. of a strong concentrated tetanus toxin was carefully inoculated into the nerve. The opening was sealed up, the cotton-wool removed, and antitoxin poured into the wound, which was then dried with sterile wool. The wound was closed. The animal was kept alive for 4 days and showed no signs of tetanus. The animal had perfect sensation in the paralysed leg.

Experiment 23.—As before, only after severing the ventral roots a longitudinal lesion was made in the lumbar cord on the same side just beyond the entry of the posterior nerve roots. Tetanus toxin was injected as before into the nerve of the paralysed side, with the same precautions. The animal developed no signs of motor tetanus or tetanus dolorosus.

Hence we must conclude that the posterior root ganglion behaves to tetanus toxin in the same way as it does to colloidal dyes and acts as a barrier to the spread, and for this reason tetanus dolorosus does not occur with subcutaneous inoculation or infection.

D. The Action of Iodine on Tetanus Toxin.

It is generally held that the exposure of tetanus toxin to iodine renders it toxoid, that is, renders it incapable of producing the symptoms of tetanus on inoculation, subcutaneously or intravenously, but capable still of provoking antibody formation.

Now if we agree with Ehrlich that the antibody is formed, especially by the tissue specifically affected by the toxin, we should have to believe that the iodised toxin reaches the central nervous system in the same way as the unaltered toxin does.

The following experiments are of importance both in regard to the action of the iodine on the toxin and the seat of antibody formation.

In these experiments the toxin was exposed to the action of an equal part of Gram's iodine solution for periods of 1 hour and more.

Subcutaneous Inoculation of Iodised Toxin.

The M.L.D. of the toxin subcutaneously for a mouse (of 10 grms.) was 0·0001 c.c.

0·25 c.c. of the iodised mixture (1 hour's exposure) = 0·125 c.c. of the original toxin had no effect on a mouse when inoculated subcutaneously. To ensure complete removal of the iodine, the mixture was shaken up with cod-liver oil, and the clear fluid was tested with the same result.

1 c.c. of the mixture produced no effect on subcutaneous inoculation into a guinea-pig of 180 grms. These experiments show that the iodine had so affected the toxin that subcutaneous inoculation no longer produced any symptoms, and further that the endeavour to remove any possible excess of iodine by Ol morrhuæ in no way affected this. This was important, since it was desired to eliminate the possibility of any free iodine acting on the nerves, etc., in the next experiments.

Experiment 24.—Rabbit anæsthetised under ether, aseptic operation. Both sciatic nerves were exposed, and 0·25 c.c. of the above iodised toxin inoculated into each. No result occurred, whereas 0·25 c.c. of the same toxin (*vide supra*, Experiment 19) produced typical tetanus on the inoculated side.

Intracerebral Inoculation of Iodised Toxin.

Experiment 25.—A guinea-pig of 350 grms. was anæsthetised with ether, trephined with aseptic precautions, and 0·1 c.c. of the above toxin-iodine mixture inoculated deep into the brain. The wound was then sewn up. The animal developed typical cerebral tetanus in 3 hours, and was then killed.

Experiment 26.—To compare the toxicity of iodised toxin (1 hour's exposure) and unaltered toxin on intracerebral inoculation. The iodised toxin was prepared as above. Six large guinea-pigs of about equal weight were anæsthetised, trephined, and the following doses of toxin (iodised and ordinary) inoculated deeply intracerebrally as follows; allowance being made for the dilution with iodine:

	Iodised Toxin.	Ordinary Toxin.
0·0001 c.c.	Nil.	Nil.
0·001 ,,	Nil.	Nil.
0·01 ,,	Typical symptoms in 8 hours.	Typical symptoms in 10 hours.

Thus the toxic dose of iodised toxin is about the same as for the unaltered toxin (slight differences in site of inoculation allowed for). Hence it must be concluded that in some way the toxic molecule becomes so affected that it is no longer carried up the axis cylinders to the cornual cells, and that which may travel along the neural lymphatic sheaths cannot diffuse through to the anterior cornual cells; but that iodised toxin inoculated directly into the central nervous system is as potent as it originally was. It might be argued that the exposure of the toxin to the iodine was not long enough and the symptoms were due to toxin which had been insufficiently acted on. If this were so, it is peculiar that the hour's exposure produced no alteration in the M.L.D.

To test this further, mixtures of toxin and iodine were exposed at 37° C. for 3 hours and 6 hours, and the results were as before, 0·01 c.c. produced typical symptoms in 10 hours in large guinea-pigs. The other possibility is that the affinity of the brain lipoid is sufficient to absorb all the iodine and so free the toxine which has not been sufficiently long acted upon. Against this is the fact that exposure to Ol morrhuæ for long periods does not bring back the toxicity of tetanus toxin as evidenced by subcutaneous inoculation.

From these experiments we must conclude that the iodine combining with the toxic molecule prevents it from getting to the anterior cornual cell, either—

- (*a*) Preventing its ascent along the axis cylinder.
- (*b*) Preventing diffusion into the anterior cornual cell of any that may have travelled to the cord along the neural lymphatic paths.

Further, they show that, since iodised toxin is used for the production of tetanus antitoxin, and since direct intracerebral inoculation of the iodised toxin produces typical cerebral tetanus, and has no effect when inoculated subcutaneously, the antitoxin must be produced by the tissues other than the central nervous system.

E. SITE OF ACTION OF TETANUS ANTITOXIN.

(*a*) *Can the Antitoxin pass through the Capillaries of the Central Nervous System to the Affected Areas?*

Meyer and Ransome (1903 [2]) endeavour to solve this problem by comparing the neutralising value of equal amounts of cord from a normal and passively immunised animal. They are unable to solve the question. This method of examination is difficult, since—

1. The normal central nervous tissues `can neutralise tetanus toxin.
2. Antibodies on injection go into combination with the tissues in some other state, and the extracts of or the ground up

tissues containing them do not react like the original antibodies *in vitro*, and probably react differently *in vivo*.

Tetanus antitoxin is usually obtained by immunising horses, hence the antitoxin cannot get into the central nervous system unless the horse serum can get there. We therefore tried the anaphylactic method of demonstration of the presence of horse serum in the central nervous system.

Experiment 27.—A series of guinea-pigs of about 250 grms. were inoculated intravenously with 5 c.c. of normal horse serum. They were killed at intervals, washed out as white as possible with sterile normal saline. The tissues were ground up and injected into guinea-pigs of 180 grms. After 3 weeks these pigs were tested to see if they had become sensitised to horse serum.

	Guinea-Pigs inoculated with Tissues from Animals kept alive for			
	12 Hours after Inoculation of the Horse Serum.	24 Hours after Inoculation of the Horse Serum.	72 Hours after Inoculation of the Horse Serum.	6 days.
Brain and cord .	Nil	Nil	Nil	Nil.
Liver . .	Acute anaphylaxis, death in 3 minutes	Acute anaphylaxis, death in 3 minutes	Acute anaphylaxis, death in 3 minutes	Slight.
Spleen . .	do.	do.	do.	Nil.
Omentum .	do.	do.	do., death in 30 minutes	Nil.

Now these experiments prove that horse serum does not get into the central nervous system. The only possible objection to the experiment may be that the washing out completely removed any horse serum in the tissue spaces of the cord.

Experiment 28.—A further experiment was performed. Two guinea-pigs of about 250 grms. were inoculated intravenously with 5 c.c. of horse serum, killed after 12 and 24 hours, bled, and the brain and cord removed without first washing out. The result was as above.

Experiment 29.—Same as 28, only the animals kept alive 2 and 6 hours respectively. The brain and cord of these were incapable of sensitising guinea-pigs to horse serum.

Now the anaphylactic reaction is a very sensitive reaction, as little as 0·00001 c.c. of horse serum being required to sensitise an animal (guinea-pig). Hence we must conclude that tetanus antitoxin does not reach the central nervous system by way of the blood vessels.

2. *The Question of the Passage of Antitoxin from the Cerebro-spinal Fluid into the Cord.*

This deals with the question of intrathecal inoculation of tetanus antitoxin for the treatment of tetanus. In order to elucidate this point the following experiment was performed.

Experiment 30.—A large rabbit was anæsthetised with ether, the dura was carefully exposed in the lower dorsal region, and two thick soft ligatures were drawn around the dura, gently tightened, and a drop of tincture of iodine inoculated into the space between the two ligatures in order to cause adhesions to the dura. The animal recovered well, but had some weakness of the legs. A week later 2·5 c.c. of tetanus toxin were inoculated into the right hind-leg. The animal developed typical tetanus in 36 hours. The animal was anæsthetised with ether, the dura carefully exposed in the upper lumbar region, pinched up free from the cord and ½ c.c. of concentrated tetanus antitoxin (500 units) injected into the sub-arachnoid space. The wound was sewn up and the animal recovered well. No alteration occurred in the tetanus of the affected side for 7 days, when animal was killed.

It was found that there were firm adhesions of the dura to the cord in the dorsal region, so that the tetanus antitoxin had been retained for a long period in the lumbar region.

Experiment 31.—A large rabbit was inoculated with 2·5 c.c. of tetanus toxin into the left hind-leg. It developed a typical tetanic rigidity of that limb in 48 hours. The animal was then anæsthetised, the sub-cerebellar space exposed, and as much cerebro-spinal fluid as could be was removed by inverting the animal. Then the animal was held vertically with the head up and 2 c.c. of very concentrated tetanus antitoxin (10 c.c. concentrated to 2 c.c.) was inoculated into the sub-arachnoid space. The animal was kept like this for some minutes, and afterwards it was kept with the body sloped so that the head was high and the pelvis low.

The rigidity persisted for 7 days when the animal was killed. There were joint changes in this and in the last experiment.

Experiment 32.—2·5 c.c. tetanus toxin were inoculated into the right leg of a large rabbit. Typical marked tetanus developed in 48 hours. The animal was then anæsthetised with ether, the dura carefully exposed in the lumbar region, and 0·5 c.c. of concentrated toxin inoculated into the lumbar sub-arachnoid space. The wound was sewn up, and the animal was quite well, but the tetanus remained unchanged in the limb.

Hence there is no evidence of the penetration of the antitoxin from the cerebro-spinal fluid to the cord in the rabbit.

The method of testing for the presence of horse serum in the cord by sensitisation of the guinea-pig was not performed, since the injection of a foreign serum always produces some local reaction of the meninges, and it is certain that the protein would be held by the cells of the meninges in sufficient quantity to sensitise a guinea-pig.

3. *Tetanus Antitoxin inoculated Intraneurally.*

Meyer and Ransome (1903 [2]) state that if tetanus antitoxin be inoculated into a nerve it blocks the passage of the toxin up that nerve, also if toxin be inoculated intravenously the injection of tetanus antitoxin into a nerve protects the corresponding anterior cornual cells and so the area supplied by that nerve.

Dealing with these statements in order :—

1. The injection of tetanus antitoxin into the sciatic nerve prevents the occurrence of tetanus in that limb, or at least greatly delays the onset and diminishes the severity of the symptoms (pp. 374, Experiments 2 and 3; p. 375, Experiment 4). We have been able to get exactly the same results.

Experiment 33.—A rabbit was inoculated with 0·25 c.c. of tetanus antitoxin (250 units) into the right sciatic nerve. Anæsthetic ether. Then 2·5 c.c. of tetanus toxin was injected subcutaneously into each leg.

The left leg showed typical tetanus in 48 hours, the right one showed slight symptoms in 96 hours.

2. Intraneural antitoxin protects against cord spread.

Experiment 34.—Similarly to Experiment 5 of Meyer and Ransome, p. 375, protection can be obtained locally by intraneural inoculation of antitoxin in generalised tetanus.

A large rabbit was anæsthetised. The right sciatic was exposed, and $\frac{1}{4}$ c.c. of concentrated (250 units) of antitoxin inoculated into it.

The rabbit was then given 2·5 c.c. of tetanus toxin (0·0001 = M.L.D.). In 36 hours there was marked cephalic tetanus; in 48 hours the fore-limbs were markedly tetanic, the hind-limbs quite free. In 72 hours the left hind-limb showed some rigidity, the right was quite free; the animal died next day, the right limb being quite free to the time of death.

Hence tetanus antitoxin appears to protect locally when injected intraneurally against toxin spreading along the cord, as well as against that travelling up the nerve. In this connection our previous experiments (*vide supra*) must be borne in mind where normal horse serum or egg albumen produced a great delay in the onset and a diminution in the severity of the local symptoms when inoculated intraneurally. Again, the following experiment is interesting :—

Experiment 35.—A rabbit was anæsthetised with ether, the left sciatic nerve was exposed, and some iodine was injected into it high up. The animal had no weakness in the limb. Ten days later the animal was inoculated subcutaneously in the left front-paw with 5 c.c. of potent toxin. The animal developed typical tetanus of that limb in 24 hours, trunk and cephalic tetanus in 48 hours, in 60 hours the right hind-limb was affected, but even after 96 hours the left hind-limb showed no sign of tetanus.

Thus simple blocking of the lymphatics of a nerve also acts in greatly delaying the spread of tetanus to the limb supplied by the nerve whose lymphatics have been blocked. This makes it appear as if the toxin which gets into the general circulation and so gets to other nerves peripherally, plays a great part in the march of tetanus as well as the direct spread in the cord mentioned by Meyer and Ransome, since in this experiment the prevention of this factor locally hindered the occurrence of tetanus in the limb whose nerve was so treated.

It was next proposed to solve the question, if the antitoxin really ascends the nerve to the cord and thus influences an already affected part, by inoculating horse serum into the nerve and testing the nerve and cord for foreign albumen by sensitisation tests.

Experiment 36.—Accordingly a rabbit was anæsthetised, the left sciatic nerve exposed near the middle of the thigh, and 0·5 c.c. of horse serum was inoculated into it. After 24 hours the animal was killed, washed out with saline, the nerve from above the site of injection to the intravertebral foramina was ground up and injected intravenously into a guinea-pig of 180 grms., and the lumbar cord and nerve roots were similarly inoculated into another guinea-pig. After 3 weeks the guinea-pigs were tested for hyper-sensitiveness to horse serum.

Guinea-pig inoculated with cord, etc. . .	Nil.
,, ,, ,, nerve . .	Convulsions, paresis, long delayed recovery.

Experiment 37.—The same experiment, only animal kept alive for 48 hours.

Guinea-pig inoculated with nerve . .	Convulsions, recovery.
,, ,, ,, cord . .	Nil.

Hence from these experiments it does not appear that the horse serum travels far along the nerve, and it makes it more probable that by blocking the neural lymphatics with antitoxin, the toxin ascending from the periphery is neutralised, and so the summation of the action of toxin ascending the nerve and that spreading in the cord from other parts is prevented, with consequent diminution of symptoms.

Experiment 38.—The following experiment with tetanus antitoxin also shows that the intraneural antitoxin cannot influence anterior cornual cells already affected.

A rabbit received 2·5 c.c. of tetanus toxin into the left hind-leg. Twenty-four hours later when tetanus had commenced it was anæsthetised with ether, and 250 units of tetanus antitoxin (concentrated) were inoculated into the right sciatic nerve at the level of the upper part of the thigh. The rigidity in the limb was in no way diminished but became more marked. There was, however, no spread to other limbs.

Experiment 39.—A rabbit received 4 c.c. of tetanus toxin in the left thigh. Twenty-four hours later when there was marked rigidity, the animal was anæsthetised and 250 units of antitoxin (concentrated) inoculated into the right sciatic nerve high up. This in no way influenced the tetanus of the left limb; there was, however, no further spread.

In all these experiments it seems justifiable to conclude that tetanus antitoxin is not carried to any degree to the cord either by the axis cylinders or neural lymphatics when inoculated directly into the nerve, but that its sole action when thus inoculated appears to be the local neutralisation of the toxin coming up the neural lymphatic sheaths. This appears to be further evidence that tetanus toxin travels in the neural lymphatic sheaths, hence we would conclude that antitoxin acts upon the toxin in the circulation and tissues, and prevents its spread from thence into the nerves and central nervous system, and that it has no effect on the toxin already travelling along the nerve and that in the central nervous system

SUMMARY.

The experiments recorded in this paper show—

1. That although tetanus toxin ascends to the central nervous system by way of the axis cylinders of the nerves, it also to a very great extent passes up the nerves to the cord by way of the perineural lymphatics. Blocking of the latter paths greatly delays and in some cases completely prevents the occurrence of tetanus in the part corresponding to the nerve whose lymph path has been blocked.

2. Although tetanus toxin passes rapidly from the blood vessels into the connective-tissue spaces and thence to the thoracic duct, the toxin does not pass from the capillaries of the central nervous system to the tissues thereof.

3. Tetanus toxin does not pass from the choroidal plexus to the cerebro-spinal fluid.

4. Although bacteria can pass through the posterior root ganglion to the cord, colloidal pigments and tetanus toxin are prevented from doing so.

5. Iodine, although it prevents tetanus toxin from producing its characteristic effects when iodised toxin is inoculated subcutaneously or intravenously, does not affect the toxin when inoculated intracerebrally, it does not hinder the occurrence of the typical symptoms of cerebral tetanus, and there is no apparent diminution in its toxicity.

6. Tetanus antitoxin does not pass to the central nervous system either by way of the blood vessels, axis cylinders, or neural lymphatic channels. It also cannot pass from the cerebro-spinal fluid when inoculated intrathecally into the substance of the cord. The antitoxin simply acts by combining with the circulating toxin, and that at the seat of production, and prevents it from reaching the central nervous system. The toxin already in this position is unaffected.

REFERENCES.

1. TEALE AND EMBLETON . . *Proc. Roy. Soc. Med., London*, 1914, vol. v. pp. 69–96.
2. MEYER AND RANSOME . . *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1903, Bd. xlix. Ss. 367, etc.
3. ORR AND ROWS . . . . *Edin. Med. Journ.*, 1916, vol. xvii. pp. 78–89.
4. GOLLA . . . . "Report to the Tetanus Committee," War Office, 1917.
5. MARIE AND MORAX . . *Ann. de l'Inst. Pasteur*, Paris, 1902, tome xvi. p. 818.
6. BRUSCHETTINI . . . . Quoted by Meyer and Ransome, *vide supra* (2), p. 372.
7. METCHNIKOFF . . . . *Ann. de l'Inst. Pasteur*, Paris, 1898, tome xi. p. 89.
8. HOMEN . . . . . . "Arb. aus dem Path. Institut zu Helsingfors," 1905, Heft 1–2, Bd. i. S. 12.

3/

[*Reprinted from* "*Heart*,"
Vol. VI, *No.* 1, *April*, 1915.]

PAROXYSMAL HEART-BLOCK WITH PAROXYSMAL AURICULAR FIBRILLATION.

By K. DOUGLAS WILKINSON and H. G. BUTTERFIELD.

The patient, aged 69, was admitted to hospital in November, 1912, complaining of blood-spitting and pains in the chest of seven months' duration. He said that until the age of 67 he had enjoyed very good health, but for two years he had suffered from bronchitis and "giddy turns." The family history was good ; he had taken very little alcohol and was a non-smoker. He had attended his work (that of a brass founder) until shortly after the blood-spitting began, but then, owing to frequent attacks, shortness of breath and general weakness he had to give it up. The pain first began in the inter-scapular region but later moved to the front of the chest. It had been very severe but at the time of admission was slight. His weight had diminished considerably since the commencement of the illness.

Condition on admission. The patient was a thin and pale old man, easily rendered dyspnœic, even by the exertion of speaking. His cough was frequent ; with a muco-purulent sputum containing streaks of bright blood. The chest was large and symmetrical but movement of both sides was impaired, especially of the left. All over this side the percussion note was poor and at the base there was definite consolidation. The heart was obscured by the lungs and no impulse could be felt. The heart sounds were feeble but clear, except in the aortic area where a short rough systolic murmur was heard. There was nothing to direct any special attention to the heart until November the 10th. On the morning of that day, after his chest had been examined, he sat up in bed, put on his shirt and lay down again. It was then noticed that no pulse could be felt at the wrist although a few minutes previously it had been full (90 per minute). On looking at his face he was seen to be more pallid than usual ; he then closed his eyes, lost consciousness, and had a typical Stokes-Adams attack. In this he made some convulsive movements of the legs and arms and his face twitched slightly. The convulsions lasted about ten seconds before the pulse returned and the duration of the whole attack was, perhaps, half a minute. When the pulse did return it was very slow, and after some ten beats stopped again, when another attack commenced. After these attacks the pulse remained at a rate of about thirty-six per minute for twenty minutes and fortunately a tracing was obtained showing the return to the normal (Fig. 1). Thereafter the pulse remained normal in frequency and rhythm until the following day. With regard to this attack the patient stated that he had never suffered from a similar condition, but thought that he had fallen asleep. He was not in the least alarmed and said that the "giddy turns" were not at all like this attack.

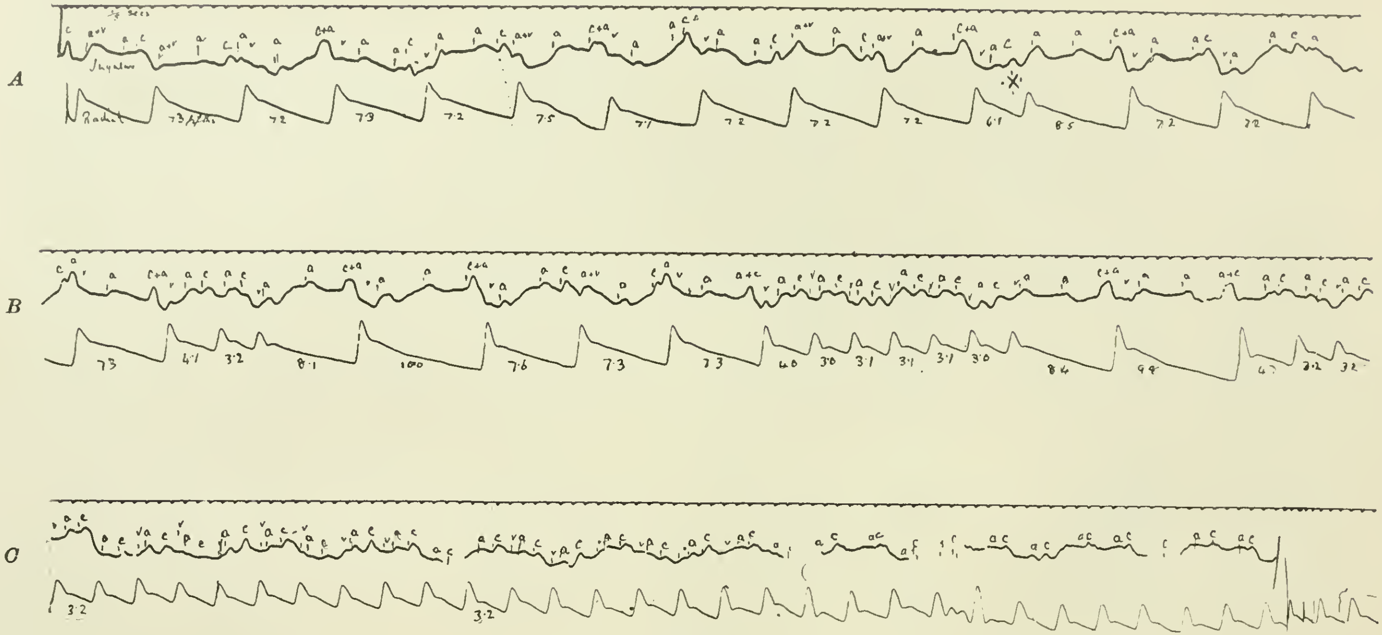


Fig. 1.

On the following morning he was again examined, and after putting on his shirt had two more attacks similar in every respect to those described. On this occasion the pulse remained slow for about four hours after the attacks. From November the 10th to February the 17th the pulse was taken every hour in order to note any variation in rate and rhythm. Tracings taken on several occasions showed that the intermissions were due to dropped ventricular beats occasioned by impairment of conduction from auricle to ventricle and that the irregularity was due to auricular fibrillation.

The following is a summary of the dates and duration of the variations noted as "intermittent" or "irregular." It is probable that there were abnormal periods of short duration which were not noticed, but these cannot have been frequent, as the patient was distinctly uncomfortable during the paroxysms of auricular fibrillation. When the ventricular contraction failed three or four beats were dropped in each minute and no heart sounds could be heard corresponding in time to the dropped beats. On all these occasions (with one exception) the patient was seen by one of us (K. D. W.), and on eight occasions tracings were obtained.

DATE.	DURATION OF ATTACK.	PULSE RATE.	CONDITION PRESENT.
Nov. 10th	30 minutes	36	Complete heart-block
,, 11th	4 hours	36	Complete heart-block
,, 12th	2½ hours	112	Auricular fibrillation
,, 16th	1 hour	80	Dropped beats
,, 18th	1 hour	74	Dropped beats
,, 18th	4 hours	80-116	Dropped beats and later auricular fibrillation
,, 22nd	30 minutes	82	Dropped beats
,, 28th	1 hour	104	Auricular fibrillation
Dec. 5th	1 hour	84	Dropped beats
,, 27th	30 minutes	84	Dropped beats
Jan. 18th	1 hour	100	Auricular fibrillation
Feb. 15th	2 hours	100	Auricular fibrillation (Not seen on this occasion)

The patient's general condition became worse and worse, and he died on March the 11th, 1913.

Tracings. Fig. 1 is divided in three parts (*A*, *B* and *C*). They form a continuous polygraphic tracing and are read from left to right. Part *A* shows a condition of complete heart-block, there being no relationship between the auricular and ventricular beats. There is slight variation in the length of the ventricular cycles. At a point marked by an asterisk this rhythm is interrupted by an isolated ventricular response to the auricle; the pause following this beat is prolonged, being 8·5-fifths of a second in length. Part *B* begins with a condition of complete heart-block, but early in the curve there are two responses to the auricle; there follow two longer beats before the ideo-ventricular rhythm regains its former frequency. Three beats later there is a series of six rapid beats, obviously responses to

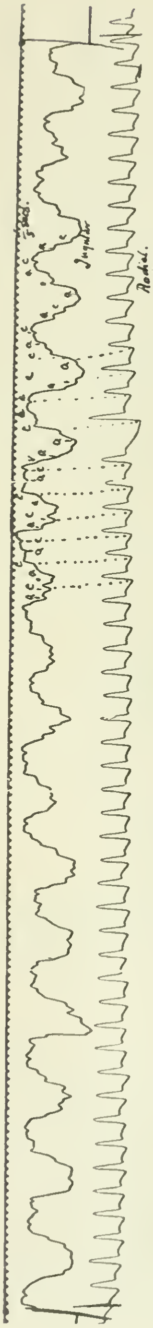

⅕ secs.
Jugular
Radial
a
c
v

Fig. 2.

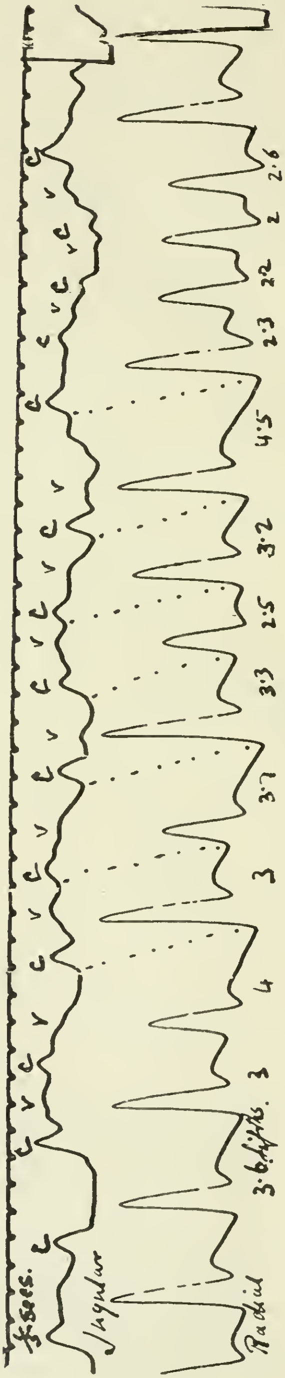

⅕ secs.
Jugular
Radial
c
v
3.6 lifts.
3
4
3
3.7
3.3
2.5
3.2
4.5
2.3
2.2
2
2.6

Fig. 3.

the auricle ; these are followed by two long beats of ventricular origin and then the normal rhythm (each ventricular beat being a response to the auricle) is resumed. Part *C* shows the continuation of the normal rhythm.

The tracing thus shows the transition from a condition of complete heart-block to the normal rhythm. A curious and possibly unique feature of this tracing is the fact that whenever the ventricle responds to the auricle the "*a-c*" interval is a constant, although the heart during this period showed variations in conduction from complete heart-block to the normal rhythm. The "*a-c*" interval is only slightly longer than the normal, being in fact rather more than one-fifth of a second in duration. The prolonged ventricular cycles after responses to the auricle appear to lend support to the suggestion that the ventricular pacemaker is readily fatigued by rapid ventricular beats. Cushny (this *Journal, Vol. III*) obtained these prolonged cycles in experimental heart-block. He found that a series of induced beats in an isolated ventricle led to a slowing of the ideo-ventricular rhythm.

Fig. 3 shows a regular heart beat with a normal sequence of chambers. There is one dropped beat in the radial curve followed by some alternation. The pause might be due to an extra-systole of the ventricle so weak that it failed to affect the pulse, but there is no evidence of such an event in the venous curve, while the pause is too short to lend support to this view. The fact that no heart sounds could be heard corresponding to the pause makes it more probable that the ventricle has for one beat failed to respond to the auricle. Fig. 4 shows auricular fibrillation.

Autopsy.

The heart was definitely enlarged and weighed sixteen ounces. There was no excess of epicardial fat on the surface, and the superficial vessels were very prominent. The *pericardium* was thickened, and at one point was roughened and shaggy. The whole of the *right side* was hypertrophied and dilated. The interior of the chambers was normal and there were no valvular lesions. There was a small patch of atheroma on the wall of the pulmonary artery about an inch away from the valves. The *left auricle* was greatly dilated. The interior was normal and showed nothing more than a blood-stained endocardium. The *left ventricle* was hypertrophied and dilated. The mitral valve showed some old thickening at the edges of the cusps, but there were no signs of recent mischief, and the remainder of the endocardium was normal. The *aortic valves* were normal except that the corpus Arantii of the left cusp appeared to be larger than those in the other two segments of the valve. The *aorta* was greatly dilated and showed extensive atheromatous change of the intimal coat. There were no gross signs of fatty degeneration of the muscle.

The left lung showed extensive consolidation and a large cavity in the lower lobe. In the upper lobe there were some more recent areas of tuberculous consolidation and scattered between these were miliary tubercles. There was no lesion in the right lung, and no further evidence of tuberculosis in the body.

Histology.

The *right auricle* was sparsely infiltrated with lymphocytes and there was an increase in the amount of fibrous tissue over a considerable portion of the muscle. The muscle fibres themselves appeared to be normal for the most part, though in one or two places a few fibres could be found which appeared to be shrunken and stained in a manner which suggested conversion into fibrous tissue. The *sino-auricular node* showed neither infiltration nor fibrosis, and in the sections examined appeared to be normal in every way. The *left auricle* showed no changes in histological character which call for comment. The *central fibrous body* showed small perivascular infiltrations in many of the sections examined and the vessels over a large area were much more prominent than usual.

The *auriculo-ventricular node and bundle* showed a series of small but quite well defined lymphocytic infiltrations which were much more frequently met with at the upper and posterior portion of the node than in any other situation. The whole of the node and bundle showed a sparse infiltration with lymphocytes, but so scanty as to be reduced to half a dozen cells in some sections. The branches of the bundle were not equally affected in that no extraneous cells of any kind were found in the right branch, while on the left side a definite infiltration was found. In addition to this the vessels in the neighbourhood of the left branch were definitely congested and the wall of the main arteriole of that region was to a great extent converted into fibrous tissue. There were many points in the node and bundle where the constituent cells of the vessel walls were evidently in a state of activity, but the occurrence of this condition was quite irregular. The microphotograph reproduced in Fig. 4 shows a typical example of the peri-vascular infiltrations at the upper limit of the node.

There was nothing in the appearance of the muscle fibres of the node which calls for comment.

The *ventricular muscle* was apparently normal as regards the fibres themselves. The fibrous tissue elements here and there were rather more noticeable than usual, but the change could not be said to amount to a definite fibrosis. The *vessels* of the heart showed a quite irregularly distributed sclerotic condition, and in a good many places the constituent cells of the walls of the smaller vessels were evidently in a condition of proliferation. The *valves* showed nothing beyond sclerosis, and no recent changes. The endocardium in general showed nothing but blood staining. Fatty degeneration could not be demonstrated histologically.

The condition of the heart may be summed up as being one of inflammation manifested by lymphocytic infiltrations which are most obvious at the upper and posterior part of the auriculo-ventricular node and by irregularly distributed changes in the vessels, unaccompanied by affection of the right branch of the bundle or by any definite alterations in the muscle fibres themselves.

SUMMARY.

A case is described which showed paroxysmal heart-block and paroxysmal auricular fibrillation.

Histological examination showed that the musculature of the right auricle was affected by fibrosis and lymphocytic infiltrations, though the sino-auricular node was not actually involved. The auriculo-ventricular node and bundle were affected by infiltrations of lymphocytes and vascular changes throughout their whole extent with the exception of the right branch of the bundle. Nothing abnormal was found in the last named part of the conducting mechanism.

We are deeply indebted to Sir Robert Simon, under whose care the patient was, for permission to publish the case.

10

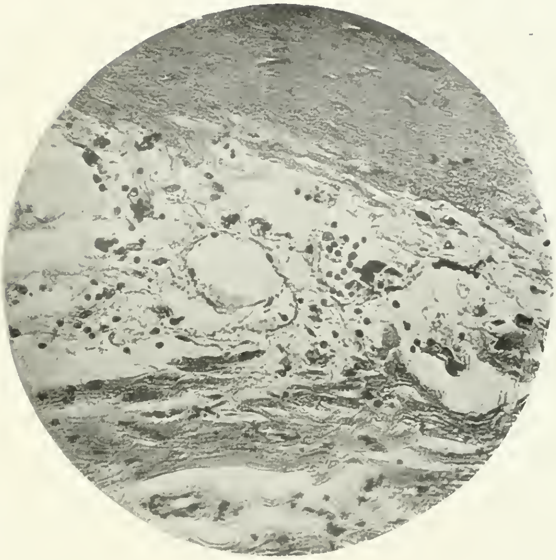


Fig. 4.

R
111
L7
v.5-6

Biological
& Medical
Serials

University College Hospital,
London. Graham Research Dept.
Collected Papers

PLEASE DO NOT REMOVE
CARDS OR SLIPS FROM THIS POCKET

UNIVERSITY OF TORONTO LIBRARY